DRUG EXTRACTION. A STUDY OF REPERCOLATION

By

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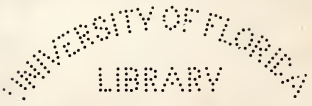

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I. INTRODUCTION

Percolation was introduced as a pharmaceutical process by Boullay and son, of France, in 1833. Between 1865 and 1870 an important modification of ordinary percolation was developed in the United States. This process, which is called repercolation, was devised by E. R. Squibb. Another modification of percolation, known as diacolation, was introduced by H. Breddin of Germany in 1930.

The research described in this dissertation comprises a study of the relative efficiencies of the U. S. P. XI repercolation process, the N. F. II repercolation process, diacolation and ordinary percolation.

II. HISTORICAL REVIEW

1. Repercolation - Boullay (1), the discoverer of drug percolation, suggested a process called "continuous displacement" for difficultly extractable substances; in this method the drug was divided in several containers and the liquid was allowed to pass successively from one to the other.

M. O. Henry (2) in his extracts from the "General Bulletin of Therapeutics" mentioned Dublanc's application of the continuous displacement process in the manufacture of syrup of pomegranate bark. The disadvantage of the Codex process of manufacture was the injury of organic extractive principles by the use of heat. The syrup produced by the process of continuous displacement was so concentrated that some

of the sugar could be left out of the formula.

M. A. Lalieu (3) brought out the first special apparatus for continuous displacement. It was composed of three or four cylinders of white cast iron 40 to 50 cm. in height and of a diameter that depended on the quantity of material to be used. Each cylinder was provided with an adjustable outlet tube, which transferred the liquid from one cylinder to the next. The apparatus was fed by water from a reservoir and when properly regulated was practically continuous, requiring little or no attention. It was said that many substances that opposed lixiviation such as rhubarb and gentian, could be better exhausted with this apparatus. It was claimed by the author that this apparatus was valuable for the extraction of drugs that allow easy passage of water, e.g. krameria, quassia, and ergot. In general it was not necessary to pack the drug as much as was customary with the use of a percolator. Cylinders of the following dimensions were used:

Height	Diameter	Quantity of Moistened Drug
45 cm.	15 cm.	2.5 Kg.
45 cm.	11 cm.	1.5 Kg.
45 cm.	7 to 10 cm.	0.5 Kg.

In the discussion of Lalieu's paper, J. Desmedt (4) emphasized the advantages of the apparatus and invited the author to study a modification of the apparatus for drugs extracted with alcohol.

In 1866 E. R. Squibb (5) became greatly concerned over the cost of manufacture of fluidextracts due to the increased price of alcohol. For this reason he suggested a process of divided percolation for

fluidextract of buchu as follows: "Divide the buchu in three equal portions. Moisten one portion with six fluid ounces of alcohol, pack it moderately in a cylindrical percolator, and pour three pints of alcohol upon it. When the last of the alcohol disappears below the surface of the powder, remove the disc of muslin or paper from the surface, and fill the percolator with water. As the percolation slackens, scrape off the upper softened layer of the exhausted powder and mix it thoroughly with water. The scraping off of the softened portion without disturbing the hard portion below is to be repeated at intervals, according to the rate of percolation, until the water becomes thick with the swollen and exhausted powder. It is then poured off and replaced with fresh water . . . until the alcohol is all pushed through, and water appears at the outlet of the percolator. Receive the percolate in four separate portions of 12, 6, 8 and 22-24 fluid ounces and set aside the first 12 fluid ounces as reserved percolate. Moisten a second portion of the buchu with a second portion of the percolate from the first percolation (the six fluid ounces), pack it in a second cylindrical percolator (or the first one readjusted) and pour upon it the third portion of the percolate from the first percolation. When this has been all absorbed by the powder, add the remainder of the percolate from the first, and when this has disappeared add first 2 fluid ounces and then 4 fluid ounces of alcohol, and then water, managing the process precisely as in the first percolation. Receive the percolate in 4 separate portions of 16, 6, 8, 10 fluid ounces and set

aside the first portion of 16 fluid ounces as reserved percolate. Moisten the remainder of the buchu with the second portion of percolate from the second percolation, and having packed in a cylindrical percolator, pour on the third and fourth portions of the percolate from the second portion in succession, and after these 8 fluid ounces of alcohol in two portions. Finally add water and proceed as in the first percolation. Receive the percolate in two separate portions of 20 and 10 fluid ounces (or the remainder) and set the last of these away to be used as so much alcohol at the next making of this fluidextract. Finally mix all the three portions of reserved percolate together and make the whole measure three pints by the addition of whatever may be wanting of that measured from the final percolate set away for the next making. This requires but one pint more of alcohol for three portions than the official process requires for one portion, diminishing the cost exceedingly."

R. W. Giles (6) described a new macerating apparatus for the more convenient exhaustion of vegetable substances with a minimum quantity of water. It consisted of a series of eight cone shaped macerators, each provided with its receiver. The water used for maceration was passed successively through the material divided among the eight cones. Each maceration was continued for such periods (varying from one to twelve hours) appropriate to the drug. The advantage of this arrangement was that with a little more water than was required to moisten the whole mass, each of the eight portions received eight successive

macerations, which was sufficient to exhaust even as stubborn a material as cinchona bark. With cinchona the exhaustion of the bark was sufficiently effected with eight and one-half gallons of water instead of eighty-four gallons which was directed by the British Pharmacopoeia.

The term "repercolation" was introduced by Squibb (7), although he claimed no credit for the idea, since infusions had been made in Spain and Portugal by passing hot water more than once through the drug, and since Boullay had used the process as early as 1833. Detailed information concerning the repercolation of the cinchonas was given.

C. L. Diehl (8) conducted some preliminary experiments in order to establish the best method for the manufacture of fluidextracts. He concluded that repercolation, which was designated by him as fractional percolation was best adapted to almost all drugs, provided that the proper menstruum was selected and provided that the whole operation was effected with knowledge and skill. He proposed to reduce the strength of fluidextracts, so that in general eight troy ounces of the drug would be represented by one pint of the fluidextract as already was the case with the fluidextracts of cinchona and wild cherry bark. He suggested a general formula which divided the powdered drug into portions of eight, five, and three troy ounces, and by which from the first eight ounces of the drug eight fluid ounces of reserve percolate were obtained, while from the second portion of five ounces, six fluid ounces of reserve percolate were collected and from the third portion of three ounces, eighteen fluid ounces of reserve percolate were collected.

W. Procter, Jr., (9) believed the fluidextracts were of correct

strengths with the exception of fluidextract of wild cherry and fluidextract of cinchona. The former could not have been made into a standard fluidextract because of the peculiar character of the process while the latter could have been made more concentrated by the use of glycerin. Although Procter expressed admiration that there was such a process as repercolation, he believed it was too complicated for official recognition.

E. R. Squibb (10) continued his studies of repercolation on cinchona, dandelion and senna by comparing the finished fluidextracts with those made by simple percolation. With cinchona, dandelion and senna the repercolation process gave nearly as strong fluidextracts as the simple percolation process. He stated that in order to prepare fluidextracts without the use of heat and without loss of menstruum, repercolation must be adopted. The various disadvantages of repercolation were enumerated by the author: (a) the process was somewhat complex and troublesome, (b) the process required skill, (c) the process required "that a stock of weak percolate of different densities be carried over from one making to the next for each fluidextract."

Next Squibb (11) in response to the solicitation of the Pharmacopoeia Committee presented his views concerning the preparation of fluidextracts. He stated the disadvantages of a process had nothing to do with its adoption because the Pharmacopoeia wanted perfect preparations in every respect. The preparation should contain all the active and useful parts of the drug in their natural condition and the inert and useless portion of the drug should be rejected. In pointing out the adverse affects of the heat necessary to concentrate the weak percolates in the ordinary

percolation process Squibb stated that: "It is not simply by the heat and oxidation of the evaporation process that all the harm is done to that portion of the preparation, but the active principles are so dissociated and split up by the concentration that they are no longer in their natural condition, but form new relations and combinations, which change their solubilities and bring a new set of reactions into play, making the preparation something else than what it professes to be." The general technique of Squibb's repercolation process may be illustrated by the following directions, which were given for the preparation of fluidextract of cinchona, using a menstruum of two parts of alcohol, one part of glycerin and two parts of water:

"Weigh the stronger alcohol, glycerin, and water in succession, in any convenient quantity at a time, into a tared bottle, and mix them thoroughly for a menstruum."

"Moisten 8 parts of the cinchona with 8 parts of the menstruum, by thoroughly mixing them, and allow the mixture to stand 8 hours in a closely covered vessel. Then pass the moist powder through a No. 8 sieve, and pack it firmly in a percolator. Pour menstruum on top until the mass is filled with liquid and a stratum remains on top unabsorbed; cover the percolator closely and macerate for 48 hours. Then arrange the percolator for an automatic supply of menstruum, and start the percolation at such a rate as to give 1 part of percolate in about 4 hours. Reserve the first 6 parts of percolate and continue the percolation until the cinchona is exhausted, separating the percolate received after the reserved portion into fractions of about 8 parts each."

"Moisten a second portion of 8 parts of cinchona with 8 parts of the weak percolate - the portion that was obtained next after the reserved percolate - and allow the moist powder to stand for 8 hours in a vessel closely covered. Then pack it moderately in a percolator, and supply the percolator automatically with the remaining fractions of weak percolate in the order in which they were received, and finally with fresh menstruum until the cinchona is exhausted. Percolate in the same manner and at the same rate as with the first portion of cinchona, and reserving 8 parts of the first percolate, separate the weaker percolate into fractions of about 8 parts each."

"Percolate the third and fourth portions of 8 parts each of the cinchona in the same way as the second portion."

"Finally mix the four reserved percolates together to make 30 parts of finished fluidextract; and having corked, labelled, and numbered the bottles containing the fractions of weak percolate, set them away until the process for cinchona is to be resumed."

"When this fluidextract is to be again made, repeat the process as with the second portion, and reserve 8 parts of the first percolate as finished fluidextract from each 8 parts of cinchona from that time forward so long as the fractions of weak percolate are carried forward with which to commence each operation."

Concerning the repercolation of cimicifuga Squibb (11) was not in general agreement with the observations of Lloyd (12). Lloyd noted that the pharmacopoeial formula for fluidextract of cimicifuga gave an inferior product. He compared the results of the simple percolation pro-

cess with the results of repercolation and found that simple percolation without maceration gave a better yield than either simple percolation or repercolation; also, that repercolation gave a smaller yield of total extractive than simple percolation. However, Squibb (11) considered cimicifuga an ideal drug for repercolation because of the resinous and oleaginous material present.

C. L. Diehl (13) answered the following query concerning fractional percolation of fluidextracts: "Does 'fractional percolation' present any advantage over simple percolation?" His conclusion based on experimental data was that if the height of the drug column were increased without increasing the diameter, nearly as good results were obtained by simple percolation as by fractional percolation. In consideration of the extra trouble of fractional percolation, the author recommended simple percolation, with the selection of tall, slender, moderately tapering percolators. In answer to a second query, Diehl stated that in his opinion fluidextracts were more concentrated than was necessary for successful medication; also, that fluidextracts were in many instances too concentrated to hold in solution all that the menstruum was capable of extracting; this belief, however, was unsupported by experimental data. As a result of this belief he recommended that more permanent fluidextracts of one-half strength be manufactured.

C. S. Hallberg (14) made a study of some of the details of fractional percolation. To prevent precipitation the point of selection of the proper menstruum was emphasized. A modified process was called by him "Simultaneous Fractional Percolation". In this process he divided the

drug in 3 or 4 equal portions and moistened according to the bulk of the drug and its fineness of powder. He macerated each portion 12 to 24 hours, and received the percolates in portions of from 20 to 40 per cent of the amount of drug used, depending upon the proportion required in the moistening. For cubeb, ergot, ginger and drugs of similar density 20 per cent was found sufficient to moisten, and to reserve. In another class were arnica, buchu, senna and most leaves and flowers that were twice as bulky as those just mentioned. These required 40 per cent of menstruum for moistening and the percolates reserved were also of that volume. Intermediate between these two classes was found a smaller group of drugs; this consisted chiefly of barks, like cascara sagrada and also a few rhizomes. But this exceptional class was quite limited in number for by far the largest portion of the drugs belonged to the other two classes mentioned.

J. W. Colcord (15) was much in favor of the process of repercolation and in his work had observed that preliminary maceration was objectionable. In simple percolation better results were obtained by him with the collection of about one-eighth of the desired amount of percolate, then alternating maceration and percolation.

A fractional percolation process was incorporated in the first issue of the National Formulary of Unofficial Preparations (16) that appeared in 1888. The process given was as follows: "Take of the drug, in powder of the prescribed fineness sixteen (16) troy ounces, and divide this in three portions, of eight (8), five (5) and three (3) troy ounces respectively. Moisten the first portion (8 troy ounces) with the menstruum and

percolate in the usual manner. Set aside the first three (3) fluidounces of the percolate, and continue until twenty-four (24) fluidounces more of percolate have passed, which should be received in several portions, so that the more concentrated will be separated from the last weak percolate. Then moisten the second portion of the drug (5 troy ounces) with the most concentrated of the percolates received during the preceding operation after the first 3 fluidounces had passed, and percolate again in the usual manner, using the several reserved percolates, successively, as menstrua. Set aside the first five (5) fluidounces, and continue the percolation until ten (10) fluidounces more have passed, which should also be recovered in several portions. Finally moisten the third portion of the drug (3 troy ounces) with the most concentrated of the last reserved percolates, and proceed as directed for the second portion. Collect the first eight (8) fluidounces separately, and mix them with the two portions previously set aside so as to make sixteen (16) fluidounces of fluid extract."

J. W. Eckford (17) reported on a pressure apparatus described by W. M. Thompson of Philadelphia. In regard to repercolation he thought it was sometimes necessary, although not usually practiced when the drug has been properly packed and allowed to macerate long enough.

Edward Moor, Jr., (18) compared percolation with repercolation in the manufacture of fluidextract of buchu and found that repercolation extracted a greater quantity of total extractive.

In 1894, F. C. J. Bird (19) considered the time was ripe to adopt the process of repercolation for at least two formulas of the B. P.:

extractum cocae liquidum and podophyllum resini. His process entailed the use of six percolators although it was found convenient to work with four.

R. A. Cripps (20) employed two types of manipulation in repercolation. The first was applicable to the preparation of fluidextracts of cimicifuga, coca, hammamelis, hydrastis, jaborandi, nux vomica, rhamnus frangula, taraxacum and viburnum. In this process the drug was divided into four equal parts. From the first part no reserve was set aside, but from the second part a reserve was set aside equal in fluid ounces to one-half the weight of the drug. From the third portion of drug a reserve was set aside equal in fluid ounces to the weight in ounces of one portion of the drug. A definite quantity of percolate from the fourth portion was mixed with the other reserves to make the finished fluidextract. In the second type the method was the same as the above process excepting that no percolates were reserved, but the entire amount of menstruum was carried through the whole series of percolators. The following fluidextracts were made by this process: belladonna, cinchona, gelsemium, glycyrrhiza, physostigma, quassia etc.

J. A. Forrest (21) described an apparatus for continuous percolation and a method for its use.

Franz Musset (22) reported on fluidextracts prepared according to the German idea of repercolation. Only two percolators were required for making from 1 to 2 Kg. of fluidextract. The author was opposed to the use of fine powders. In order to make 1000 Gm. fluidextract the amount of drug was divided into ten portions. Reserve percolate was collected

from the fourth, sixth, eighth and tenth portions of drug. In addition to the reserve, eight fractions of percolate were collected from each portion of drug and used successively in moistening and percolating the next portion of drug.

L. E. Sayre (23) reported the results of a series of experiments undertaken by his assistants on: (a) repercolation in general (b) percolation and repercolation of taraxacum. The results of these experiments show that repercolation has advantages over simple percolation, but the process was insufficient to totally exhaust the 1000 Gm. of drug in the making of fifty per cent tinctures. In the case of percolation and repercolation of taraxacum in which 631 cc. of percolate was obtained from 320 Gm. of powdered taraxacum by both processes, the results speak for the greater efficiency of repercolation. Even in this the required limit for a fifty per cent tincture was scarcely reached.

D. C. Kelly (24) investigated repercolation with the purpose of ascertaining whether or not it was possible to completely exhaust drugs in the manufacture of 50 per cent tinctures. Gentian, uva ursi and squill were selected for this investigation. He divided 100 Gm. of drug into four portions and reserved 37, 40, and 50 cc. respectively from the first three portions and from the fourth portion enough to make 200 cc. The results, which are reported in tabular form, show that repercolation did not completely extract the drugs under the conditions given.

J. V. Catford (25) attempted to simplify the process of repercolation. The apparatus consisted of a large glass tube two and one-half feet long by one and one-fourth inches in diameter cut into four equal

lengths, capable of being connected end to end by means of stoppers. Each cylinder was capable of containing 32 Gm. of moistened drug and 24 cc. of menstruum on the top. An automatic regulator at the bottom served to control the rate of percolation and to connect with the receptacles for the successive portions collected. Liquid extract of belladonna root was made by the author in the following manner: One fifth of the B. P. quantity of powdered drug was weighed in eight portions of 32 Gm. each. One was moistened with 24 cc. menstruum and allowed to macerate 6 hours before packing. After packing and sufficiently macerating, percolation was started. The first 24 cc. of percolate was used to moisten the second 32 Gm. of powdered drug which was packed in the second segment. The second segment was then placed below the first segment. When all four segments were connected in this manner the first three portions of percolate of 24 cc. each were reserved, and the menstruum supply was disconnected in order to allow the top portion to drain. This portion on removal was found to be practically exhausted. After emptying the upper segment, it was recharged with a fifth 32 Gm. portion of the drug and moistened with the fourth portion of percolate from the bottom segment and placed at the bottom of the column. In like manner the other three segments were recharged as they came to the top of the column. When all four were recharged, the next four portions of percolate of 24 cc. each were reserved and percolation was continued to exhaustion. With the exception of a short interval for the recharging of the segments, the process was practically automatic.

E. A. Andrews (26) communicated results of his experiments on belladonna with a modification of the B. P. process, which was an adaptation

of the well known process of repercolation. After using the method for several years he found it to answer admirably. The points in its favor were: (a) a batch of the liquid extract could be prepared in five days, (b) the periods of maceration were convenient, (c) a definite quantity of menstruum was used in the process, (d) the loss of spirit by evaporation during the process was reduced to a minimum, (e) the amount of alkaloid extracted was about the same as when the directions of the B. P. were strictly followed (varying slightly with the condition of the drug used), (f) the same results were obtained with large as with small quantities of the drug. The fact that the process extracted a somewhat smaller percentage of total solids was the only disadvantage; however, this was a questionable one since the extraction might vary to the extent of 2 or 3 per cent according to how the words "pack firmly" and "slow percolation" were interpreted. He stated that the results had been confirmed by A. W. Nunn.

In studies by H. V. Arny and E. M. Oxley (27) the repercolation process did not appear to give favorable results. This was judged from the fact that the repercolation of gentian did not in most cases give a product that contained as much total extractive as did simple percolation with evaporation of the weak percolates. Scoville (28) in a discussion of the paper by Arny and Oxley stated that on the basis of his experience repercolation could be used with excellent results with drugs which percolated easily as capsicum and resinous drugs in general. Remington (29), in a discussion of the same paper, said repercolation was used to a large extent by manufacturers because of the saving of alcohol; also many manu-

facturers used a proportion of 1000 Gm. drug for 750 Gm. of fluidextract, since 75 to 80 per cent of total extractive was found in the reserve percolate.

A. Azadian (30) studied the composition of fluidextracts as influenced by the mode of preparation. Percolation and repercolation were explained and sketches were given. Four sets of analytical results on ten fluidextracts were tabulated to compare those made by the author according to the Swiss Pharmacopoeia with another set made by repercolation, a third of Swiss, and a fourth of French manufacture. The drugs examined were aconite, belladonna, buckthorn, cascara, coca, kola, condurango, hydrastis, ipecac, cinchona. The fluidextracts made by repercolation showed more active principle and more total solids than those prepared by the official process by him. Repercolation was recommended because it consumed less alcohol, involved no warming, and required less time.

The repercolation process was official in the British Pharmacopoeia of 1914 (31). The process was as follows: "Take one hundred parts by weight of the drug and divide it into five equal portions. Moisten the first portion with the menstruum, set aside in a closed vessel for four hours and pack in a percolator. Add sufficient of the menstruum to saturate the drug and leave a layer of liquid above. Macerate for twenty-four hours, then allow percolation to proceed slowly, collecting the percolate in fractions of twenty parts. Moisten the second portion of the drug with the first fraction of the percolate collected. Set aside, pack in a percolator, macerate and percolate as before, using as menstruum the successive fractions of percolate collected from the portion

first treated. Again collect the percolate in fractions of twenty parts. In turn, treat in the manner described above the third, fourth and fifth portion of the drug with the fraction of percolate obtained in the percolation of the portion immediately preceding, using the successive fractions of percolate in order, until a liquid extract is obtained of the required strength."

On the appearance of the D. A-B. VI, M. Seifert (32) made a critical study of the repercolation processes adopted for the manufacture of the newly recognized fluidextracts of orange and thyme. He claimed these processes were not suitable for the small manufacturing apothecary. The processes directed the drugs to be divided into five parts and a reserve was collected from each as the process progressed. The weak percolate from the first portion was used to percolate the second, etc.

E. Bang (33) discussed the advantages and disadvantages of the various methods for the preparation of fluidextracts. A simple and reliable repercolation method, worked out by E. P. F. Petersen was described, the features of which were: (a) all heating was avoided, (b) the characteristic aroma of the drug was retained, (c) variation in the specific gravity was adjusted and (d) the procedure was quick and inexpensive as well as especially suited for small shops.

J. Schmeltz (34) studied the repercolation of fluidextracts. He pointed out that fluidextract of valerian obtained by repercolation according to Bang cannot contain the amount of alcohol required by the Danish Pharmacopoeia. A detailed discussion and criticism of Bang's method of manufacture of fluidextract of valerian was given.

M. R. Thompson (35) found Type Process B unsatisfactory for the manufacture of fluidextract of ergot, since the loss of activity on concentrating the weak percolate amounted to 20 to 30 per cent. Type Process C was found to give a more satisfactory fluidextract than the Type Process B method of the U. S. P. X., as shown by the official biological assay of the finished fluidextracts.

As an advantage of the repercolation process, it has been stated by Bennett and Cooking (36) that a weak solution of the extractive of a drug is usually a better solvent for the active constituents than the original menstruum.

L. Rosenthaler (37) made investigations of fluidextracts of cola, cinchona and belladonna by repercolation.

Z. Rektorik (38) studied a method for the more efficient extraction of alkaloids from cinchona bark by fractional percolation.

A short summary of repercolation was published by W. J. Husa and C. L. Hayck (39). It had been shown that the pioneer work of Squibb (11) was concerned with the now obsolete method of saving in storage the weak percolates from one batch of drug to the next time the fluidextract was prepared. The expense of storage, idle investment in menstruum and possibility of deterioration during storage are the chief factors responsible for the unpopularity of this method. Also it had been shown that the work of Diehl (8) (13), which was carried out during the same period as that of Squibb, dealt with a repercolation process for half strength fluidextracts. There was some agitation at that time for use of 50 per cent fluidextracts based on the claim that such fluidextracts could be

made by the retail druggist, while the adoption of 100 per cent fluidextracts would throw the preparation of fluidextracts into the hands of the large manufacturers. However, the 50 per cent fluidextracts were not adopted and hence the work of Diehl has little application to present day repercolation. In later studies by Army and Oxley (27) the repercolation process did not appear to give favorable results. In order to throw some light on the exact efficiency of the U. S. P. X repercolation process Husa and Huyck (39) conducted experiments with belladonna root and nux vomica. They found repercolation was successful for fluidextract of belladonna root and fairly successful for fluidextract of nux vomica.

P. L. Burrin and F. E. Bibbins (40) found that with a menstruum consisting of 95 per cent alcohol, fluidextract of celery fruit could be prepared by either regular percolation or repercolation. In case it was desired to manufacture fluidextract of celery fruit with use of a menstruum of 9 parts alcohol and 1 part water, as indicated in the N. F. VI, the two types of percolation were not found equally efficient. In this case repercolation gave a more satisfactory product than regular percolation.

J. Büchi and K. Feinstein (41) investigated the U. S. P. X repercolation process for the manufacture of fluidextract of cinchona. They found the process to extract only about one-half the total quantity of alkaloids of cinchona present in the drug. A time duration of 61½ hours spread over four days was required in the manufacture of one liter of the fluidextract. They concluded that the process was complicated to operate and gave no better results than the regular percolation process.

J. E. Machado and J. Sonol (42) found that fractionated lixiviation as described in the Italian Pharmacopoeia gave fluidextracts lower in extractive matter than the same fluidextracts made by the standard method of percolation.

2. New Drug Extraction Processes.

A. Repetition Diacolation.

a. Description of the Apparatus and the Process. In 1930 H. Breddin (43) described a repercolation process in which 300 Gm. of fluidextract was obtained from 300 Gm. of drug and 300 Gm. of menstruum. The drug was divided into three 100-Gm. portions and instead of using percolators, glass tubes about one-half meter in length and 3.3 cm. in width were employed. In order to obtain the total amount of percolate from each portion of the drug, water was used at the end of the percolation to displace the amount of menstruum held by the drug. This procedure was claimed by the author to be successful, since the end of the displacement was marked by the movement of a suitable floating device. The first portion of the drug was moistened and extracted with 200 Gm. of menstruum and the first 50 Gm. of percolate was reserved. The next 150 Gm. of percolate and 50 Gm. of fresh menstruum were used to extract the next 100-Gm. portion of drug. In like manner 50 Gm. of percolate was reserved from this portion and 150 Gm. percolate and 50 Gm. of fresh menstruum were used to extract the third portion of drug. From the third portion of drug 200 Gm. of percolate was reserved. The reserves were united to make 300 Gm. of finished fluidextract.

b. Advantages of the Process. The advantages of repetition diacc-

lation over simple percolation of the German Pharmacopoeia were stated by Breddin (44) as follows: (a) the process economized menstruum, (b) the process avoided distillation and the resulting contamination of large quantities of alcohol, (c) the process was more efficient than percolation, since the cylindrical tubes prevented the formation of capillary passages in the packed drug.

H. Breddin (45) claimed the advantages of the cylindrical tube over the conical percolator were as follows: (a) the cylindrical tube enabled more uniform extraction than the conical percolator because portions of the drug approximating the perpendicular mid-line in the percolator were more rapidly extracted than adjacent portions of the drug in the percolator, (b) with the cylindrical tubes the number of contacts of drug with menstruum was increased, (c) in percolators the drug was more loosely packed and absorbed larger amounts of menstruum than in the cylindrical tubes, (d) the tightly packed drug in the cylindrical tubes allowed slow penetration of the menstruum through the drug which was considered to be of value in extraction.

H. Breddin (43) and H. Trunkel (46) stated that by the avoidance of heat, diacolation produced a better tasting and smelling fluidextract than simple percolation.

H. Breddin (47) stated that the advantage of diacolation over maceration in the preparation of tinctures was the elimination of the contamination of tannin-containing drugs with the metallic press.

c. Disadvantages of the Process. H. Ihbe (48) claimed the disadvantages of Breddin's process were as follows: (a) full extraction was

not possible in the last tube, (b) the process needed close observation until the second runnings were collected, (c) difficulty was encountered in estimating the volume of the moistened drug in the selection of the correct sized tubes.

d. Drugs upon which Repetition Diacolation was Tested and the Results Obtained. H. Breddin (43) claimed he successfully extracted ergot, condurango (49), orange (50), valerian (51), male fern (52), sabadilla, cinchona, cinnamon, digitalis, and cantharides (53) by this process.

W. Rademacher (54) made fluidextract of thyme by repetition diacolation, and on the basis of active constituents present, he claimed it clearly surpassed the fluidextract made according to the German Pharmacopoeia.

F. Gstirner (55) made fluidextract of ipecac according to this process and on the basis of alkaloids and extractive matter claimed it excelled fluidextracts made by the pharmacopoeias of Germany, Switzerland, Denmark, Sweden, Great Britain and the United States.

C. J. T. Madsen (56) made thyme, valerian and senega fluidextracts by this process and on the percentages of extractive matter present concluded better fluidextracts were obtained by it than by either simple percolation or repercolation.

W. Brandrup (57) found that repetition diacolation produced a slightly better fluidextract of thyme than simple percolation.

H. Reinicke (58) found fluidextract of senega made by repetition diacolation was superior to fluidextracts made according to the pharmacopoeias of Switzerland and the United States.

e. Variations of the process. H. Ihbe (48) made fluidextracts of ergot, senega and bitter orange successfully by a modification of the repetition diacolation process.

H. Breddin (49) employed five tubes in the preparation of 1000 Gm. of fluidextract of condurango and in order to speed up the process mixed a little kaolin with the drug before packing.

In the manufacture of fluidextract of orange H. Breddin (50) displaced the menstruum with denatured alcohol.

H. Breddin (52) extracted male fern in four sections and used benzin colored red with a dye followed by water to displace the menstruum from the drug.

H. Breddin (51) mixed finely clipped cotton with valerian to assist the process of extraction.

In the manufacture of fluidextract of thyme W. Rademacher (54) evacuated the receiver, if the rate of flow was not sufficiently rapid.

H. Ihbe (48) modified the repetition diacolation process. In his procedure 600 Gm. of drug was divided into three equal portions. The first two tubes were provided with stopcocks and all three were arranged so that the percolate would flow by gravity from one to the other. The first portion of drug was moistened with 45 Gm. of menstruum, the second with 67 Gm. and the third with 88 Gm. of menstruum. From the three portions of drug the quantities of reserve percolates collected were 45 Gm., 67 Gm., and 488 Gm. respectively. The reserves were mixed to make a total of 600 Gm. of fluidextract from 600 Gm. of drug. The author claimed a one per cent solution of sodium chloride and mixtures of different

organic solvents such as acetone, chloroform, benzin, when adjusted to the same specific gravity as that of menstruum, were suitable for the displacement of the menstruum from the drug.)

H. Breddin (47) (59) described a modification of the process for the manufacture of small quantities of tinctures including (60) compound tincture of cinchona.

B. Diacolation.

a. Description of the Apparatus and the Process. H. Breddin (61) has patented in the United States an "Apparatus for Extracting Drugs and the Like" commonly called the diacolation apparatus. It was also patented in Germany (62), France (63), and Great Britain (64).

The United States Patent No. 2,046,055 (61) described the apparatus as follows: "The invention relates to apparatus for extracting drugs and the like in accordance with the method, in which the extraction fluid is slowly forced through one or several tubular vessels connected one behind the other, which vessels are filled with the comminuted drugs, so that the advance particles of fluid may enrich themselves to a very high degree with the extract substances whilst the portions of the extraction fluid, which flow through later, and which are displaced by another neutral fluid, absorb the originally remaining extract substances until the drugs are exhausted almost completely."

"The essentially novel feature of the apparatus consists in that the more or less large number of extraction tubes, which serve to receive the drugs to be extracted and which are linked up by intermediate tubular members, are connected up to a storage vessel which is associated with

devices for putting same under pressure, and in providing special auxiliary means which are interposed in a tubular connection between said vessel and the extraction tubes and which permit a very slow influx of the extraction and displacement fluid to the extraction tubes. The speed of flow is accurately controlled and the exceedingly high resistance to flow, of which the delivery of fluid is made independent, is also overcome."

A short description of the apparatus and process has been given recently by W. C. Peck (65): "The apparatus functions by driving slowly, by means of compressed air, the extracting liquid through one or more extraction cylinders. It is provided with an adjustable throttle device between the supply container for the liquid and the first or only extraction vessel, which is fitted with a dropping tube for indicating the rate of flow. Where a tincture is to be prepared only one extraction cylinder is employed, but for the preparation of extracts several cylinders in series are employed. The extraction liquid is forced from the container by means of an air pump, through the throttle device comprising a rubber tube enclosing a wick or wad of thread and clamped between plates engaged by a pressure screw. The liquid then enters a float chamber, the float of which in the raised portion closes the outlet tube. The chamber communicates with a dropping inspection chamber, the drip tube carrying a check valve such as a rubber lip valve. From this inspection chamber the liquid enters the lower end of the extraction cylinder. The cylinder is packed with powdered drug and has filtering material at the lower and the upper ends. The liquid leaves by a tube passing through a rubber plug, which may extend into a plug which, with the filter material, forms a

seal for the tube when not filled. The patent specification, however, gives no indication of how the operation of the apparatus could be conducted on the large scale."

Fluidextract of cinchona was prepared by diacolation by H. Breddin (66). A battery of nine tubes, each 80 cm. long and 1.7 to 1.8 cm. wide, was used. Six hundred grams of cinchona was moistened, macerated and packed in the tubes. By the use of the drop chamber the menstruum was allowed to enter the system at the rate of one and one-half drops a minute and was forced through the drug by a pressure not exceeding one and one-half atmospheres. The receiver was evacuated to help the menstruum penetrate the drug and to help establish the correct rate of flow. The process of making 600 Gm. of fluidextract from 600 Gm. of drug lasted about 20 days.

b. Advantages of the Process. The advantages of the diacolation process were claimed by H. Breddin (67) to be as follows: (a) some drugs, notably cinchona, were not successfully exhausted by repetition diacolation, but were successfully exhausted by diacolation, (b) diacolation provided for regulation of the rate of penetration of the drug by the menstruum, (c) diacolation provided for adjustment of rate of flow and length of drug column, (d) diacolation provided a quick, efficient and convenient method of preparing tinctures.

The United States Patent No. 2,046,055 (61) stated the advantages of the apparatus were as follows: (a) drugs were efficiently extracted with relatively small quantities of menstruum, (b) the preparation of fluidextracts was possible in one operation, (c) the numerous disadvantages of

heat were avoided.

F. Gstirner (68) stated that the advantages of diacolation over maceration in the manufacture of tinctures were as follows: (a) less time was required to make tinctures by diacolation than by maceration, (b) upon analysis tinctures made by diacolation showed a greater total extractive than tinctures made by maceration, (c) diacolation used less menstruum than maceration.

Tinctures were made by six different processes including percolation and diacolation by S. von Bari (69) and the products were compared on the basis of color, clearness and total extractive. Although percolation and diacolation extracted most of the active principles from the drug, the author concluded that diacolation gave the most promising results.

In comparing tinctures made by diacolation with tinctures made by maceration K. Höll (70) found that tinctures of nux vomica, strophanthus, cinchona and ipecac made by diacolation were much higher in alkaloidal content than the corresponding tinctures made by maceration.

c. Disadvantages of the Process. Concerning diacolation from a theoretical and practical standpoint R. Kummer (71) outlined the following disadvantages: (a) from a mathematical point of view too much emphasis was placed on the form of percolator used in the process, (b) from a survey of the literature, the author failed to see the value of pressure, (c) the difficulties involved in charging and discharging the tubes were obvious, (d) the importance of uniform packing of the drug in the tubes had been overemphasized.

E. Kessler (72) stated that in diacolation the efficiency of extrac-

tion was diminished by pressure, since it caused passages to be formed in the drug. He also stated that diacolation was not applicable to fine powders.

×C Koch (73) believed the trouble involved in the assemblage of a large diacolation battery for the manufacture of fluidextracts would hinder the adoption of the process.

In the manufacture of tinctures of cinchona and valerian by diacolation K. Höll (70) found that finely powdered drugs could not be used and that the powdered drugs should be mixed with 10 per cent of sand before packing.

Working on cinchona, J. Büchi and K. Feinstein (41) found that finely powdered drug could not be used in the manufacture of the fluidextract. They concluded the apparatus was too complex; since, for skillful operation many preliminary experiments were required.

× d. Drugs on which Diacolation was Tested and Results Obtained. In 1934 H. Breddin (66) claimed he successfully prepared fluidextract of cinchona by the diacolation process.

× Also in 1934 H. Breddin published a pamphlet (74) in which he described methods of making fluidextracts of ergot, condurango, orange, frangula, hydrastis, thyme, cinchona, senega, ipecac and valerian by diacolation. Also the theory of diacolation, description of the apparatus and the methods of making many tinctures were explained in detail.

I. Szentgale (75) found that based on the amounts of alkaloids and total extractive present diacolation with a weaker alcoholic menstruum produced a better fluidextract of hydrastis than percolation, but perco-

lation with a menstruum of the same alcoholic strength was better than diacolation in the manufacture of fluidextracts of belladonna leaves, hyoscyamus and ipecac.

In recent work J. Büchi and K. Feinstein (41) prepared fluidextract of cinchona by diacolation. They obtained no better results than by simple percolation but declined to draw any conclusions as to the efficiency of the process until after making further tests.

e. Variations of the Process. A device that prevented shutting off of the air pressure during the process of diacolation was illustrated and described by H. Breddin (76).

Likewise C. Rohman and J. Ehlers (77) introduced a piece of apparatus designed to be used for furnishing the air pressure in diacolation.

C. Mulcolation and Evacolation.

a. Description of the Apparatus and Processes. In 1934 the "Mulcolator-Kessler" apparatus (78) and the process of making fluidextracts by mulcolation was described. The apparatus consisted of six thick-walled tubes, each about 1 meter long and 6 cm. wide, connected end to end and terminated by a suction flask attached to a water pump. The apparatus was intended to make 2400 Gm. of fluidextract from 2400 Gm. of drug. The drug was divided into three equal portions and after moistening the first 800 Gm. of drug with 400 Gm. of menstruum and macerating for 12 hours, it was packed in tubes one and two. Menstruum was then run into the tube at the rate of 20 to 25 drops a minute, and 400 Gm. of percolate was collected in the suction flask attached at the end of tube two. The 400 Gm. of percolate collected was used to moisten the next 800 Gm. of drug, while

from tubes three and four 200 Gm. and 400 Gm. respectively of reserve were collected. Another 400 Gm. portion of percolate was collected from tube four for moistening the next 800 Gm. of drug and from tube five 400 Gm. and tube six 1400 Gm. of percolate were reserved. The 200 Gm. from tube three, 400 Gm. from tubes four and five, along with 1400 Gm. from tube six made a total of 2400 Gm. finished fluidextract from 2400 Gm. of drug. The menstruum was displaced from the extracted drug by water.

In 1935 E. Kessler (79) described the process of evacolation in which a glass tube called the evacolator was used. In all cases the tube was about one meter long, but the diameter was varied according to the volume of drug to be extracted. The form of drug best suited to the process was a moderately coarse powder containing all the finer particles. Since, according to the author, one purpose of the preliminary moistening was the removal of air; moistening was not considered necessary in the process of evacolation.

The process that was applied to the manufacture of fluidextract of condurango was outlined by E. Kessler (80) as follows: Two hundred and fifty grams of dry moderately coarsely powdered condurango was packed in a tube 1 meter long and 28 mm. wide. The menstruum was supplied at the top of the tube by a vessel provided with a device for adjusting the delivery. A suction flask was attached to the tube and the apparatus was evacuated by means of a water pump. The menstruum was allowed to flow in the tube on the drug at the rate of 2 drops a minute until the drug was entirely penetrated by it. The valve between the water pump and the suction flask was then closed and the percolation was carried out at the

rate of about 2 drops a minute. When percolation ceased water was let in from the supply vessel at the same rate until the menstruum was entirely displaced from the drug. In order to collect 250 Gm. of percolate about 36 hours was required.

b. Advantages of the Processes. It was claimed that the mulcolation apparatus (78) arranged in a circular manner had the advantage of taking up less space than the diacolation apparatus.

E. Kessler (79) claimed the following advantages of evacolation over the present official simple percolation and maceration methods for making fluidextracts and tinctures: (a) fluidextracts were rapidly prepared at low cost, (b) the disadvantages of heat were avoided, (c) all contact of the preparation with metal was avoided, (d) the simplicity of the process.

Evacolation required a much simpler and less costly apparatus than diacolation and it operated without the difficulties of diacolation. These were the advantages of evacolation over diacolation given by J. Büchi and K. Feinstein (41).

In a recent article by E. Kessler (81) the advantage of the use in evacolation of a finer powder than in diacolation was brought out.

c. Disadvantages of the Processes. H. Breddin (82) claimed the disadvantages of mulcolation were as follows: (a) the suction applied in mulcolation damaged the extraction, (b) the mulcolation apparatus could not be used to make small quantities of fluidextracts, (c) the mulcolation principle overlooked the fact that the extraction of some drugs required more tubes or a longer column than the extraction of others.

After the appearance of the mulcolator H. Breddin (72) stated that

the mulcolator was more complicated than the simple apparatus required in repetition diacolation.

d. Drugs upon which Mulcolation and Evacolation were Tested and Results Obtained. Good results were obtained in the use of the mulcolation process by Zimmerman (83).

Fluidextract of condurango was made by the process of evacolation by E. Kessler (80) and he claimed a better fluidextract was produced than by the official simple percolation process.

W. Brandrup (84) extracted belladonna leaves by evacolation. Since 2.31 Gm. of the 2.60 Gm. total alkaloids present in 500 Gm. drug was obtained by evacolation, the author considered the process satisfactory.

Working on several widely different types of drugs C. J. Blok and H. J. A. ter Wee (85) prepared fluidextracts by evacolation. Based on the active constituents present in the finished preparations, better fluidextracts were claimed to be made by evacolation than by diacolation or by percolation.

× J. Büchi and K. Feinstein (41) employed the evacolation process on previously moistened and macerated cinchona. The resulting fluidextract was lower in alkaloids and extractive matter than fluidextract of cinchona made by simple percolation.

By a slight modification of the evacolation process E. Kessler (81) made fluidextract of chamomile with good results.

× e. Variations of the Processes. By a slight variation of the evacolation process in the use of previously moistened and macerated cinchona instead of the dry drug, evacolation was used by J. Büchi and K.

Feinstein (41). Analysis of the fluidextract for alkaloids and extractive matter showed the preparation was greatly inferior to the fluidextract of cinchona made by simple percolation. By displacing the drug with regular menstruum instead of water, and by using cinchona in the form of a slightly coarser powder, a fluidextract was produced that contained as much alkaloids and extractive matter as simple percolation and more than fluidextracts made by either diacolation or repercolation.

In a recent article by E. Kessler (81) fluidextract of chamomile was prepared by a slight variation of the evacolation process. One thousand grams of drug was divided into two equal parts. The first 500 Gm. was placed in a tube of convenient size and evacolated. One thousand grams of menstruum was allowed to run in the tube on the drug at the rate of 5 drops a minute and 1000 Gm. of percolate was collected in two 500 Gm. portions. Water was used to displace the remaining menstruum from the drug. After discharging the tube, the second 500 Gm. portion was extracted in the same manner as the first portion of drug and the menstruum was displaced by water. On the basis of color, the author claimed the fluidextract produced by this process was of good quality.

3. The Extraction of Belladonna Root.

A review of the literature concerning the extraction of belladonna root up to 1934 was incorporated in the thesis "The Effect of Fineness of Powder and of Variation in Solvents on the Percolation of Belladonna Root", by the author (86).

From 1934 to the present date several articles concerning the extraction of belladonna root have appeared in the literature. L. Rosenthaler (37) made an investigation of the repercolation of belladonna

root. According to W. Brandrup (84) L. Rosenthaler extracted by repercolation only 44.07 per cent of the total alkaloids present in belladonna root.

W. J. Husa and C. L. Huyck (87) investigated the effect of fineness of powder and of variation in solvents on the percolation of belladonna root. One hundred gram portions of drug in the form of very fine (No. 80), fine (No. 60), moderately coarse (No. 40), and coarse (No. 20) powders were percolated with the U.S.P. X fluidextract of belladonna root menstruum consisting of a mixture of 5 volumes of alcohol and 1 volume of distilled water. A reserve percolate of 80 cc. and several successive 100 cc. portions of weak percolate were collected from each portion of drug. The various fractions of percolate were analyzed for content of alkaloids and extractive matter. Although the moderately coarse (No. 40) powder gave the largest yield of alkaloids in proportion to extractive matter, the differences shown by duplicate analyses for alkaloids were smaller than the average experimental error. Therefore, the authors concluded that within the limits of a coarse powder (No. 20) and of a very fine powder (No. 80), the fineness of powder was of minor importance in the extraction of belladonna root. Also, from a series of percolation experiments on belladonna root using various alcohol and water mixtures, it was found that proportions of alcohol and water ranging from alcohol 5 volumes-water 1 volume to alcohol 1 volume-water 1 volume gave the best results.

J. Husum (88) prepared extract of belladonna from the leaves or root by fermentation. One kilogram of root or leaves was boiled with 4 or 5

kilograms of water. The decoction was set aside until it was acid to litmus and covered with a mould growth. It was claimed that starches, sugars and mucilaginous substances were removed from the decoction by fermentation, while the alkaloids formed salts with the organic acids present in the decoction. The decoction was strained and filtered, but the residue obtained was again macerated for 12 hours, restrained and refiltered. Finally the liquid extract was boiled to remove waxes and albumins, and concentrated in vacuo to a soft extract. One kilogram of the root yielded about 330 Gm. of soft extract.

The effect of the degree of comminution on the percolation of belladonna root was studied by A. W. Bull (89). One hundred and twenty gram portions of moderately coarse, moderately fine, and finely powdered belladonna root were percolated with a menstruum of 7 volumes of alcohol and 1 volume of distilled water, and several percolates of 50 cc. were collected from each portion of drug. Apparently an optimum degree of comminution existed for the extraction of the total solids of belladonna root. When the ratio of the amounts of alkaloids to extractive matter was considered, a moderately coarse powder gave a greater yield of alkaloids than either the finely or moderately finely powdered drug. From the amounts of alkaloids present in the various fractions, it was shown that the alkaloids of the drug were more easily extracted than the other extractable material present.

In 1935 W. J. Husa and C. L. Huyck (39) extracted belladonna root by the U.S.P. X repercolation process. Since the alkaloidal content of the different reserve portions and finished fluidextract exceeded the official

requirements, the U.S.P. X repercolation process for making fluidextract of belladonna root was found efficient.

III. EXPERIMENTAL DATA

1. Scope and General Methods - In order to compare the efficiency of various extraction processes, fluidextracts of belladonna root were prepared by a number of different methods. The relative efficiency of the different processes was determined by comparing the finished fluidextracts as to content of alkaloids and extractive matter and by noting the time and labor required in each case. The extraction processes studied were as follows: simple percolation, repercolation, modified repetition diacolation and modified diacolation. In some instances various fractions of percolate were analyzed in order to throw further light on the progress of the extraction at various stages. In connection with this work a study was made of the relative efficiencies of the different forms of percolators used in the extraction of drugs.

2. Materials Used.

a. Drug - One hundred pounds of moderately coarsely powdered belladonna root was purchased from S. B. Penick and Company. To insure uniformity throughout the experimental work, the drug was thoroughly mixed. The results of numerous assays of the drug according to the U.S.P. XI method gave an average of 0.484 per cent of the alkaloids of belladonna root.

b. Menstruum - The menstruum used throughout the experimental work was a mixture of 4 volumes of alcohol and 1 volume of distilled water as

prescribed in the U.S.P. XI for making fluidextract of belladonna root.

3. Methods of Analysis.

a. Alkaloids - The regular U.S.P. XI assay process for fluidextract of belladonna root was used.

b. Total Extractive - Ten cubic centimeters of the fluidextract was evaporated to dryness on a water bath and placed in an electric oven at 105 degrees centigrade until the difference between two successive weighings was not over ten milligrams.

c. Specific Gravity - Duplicate determinations of the specific gravity of each fluidextract were made by the pyknometer method and an average of the two determinations was reported. In each specific gravity determination a weight of a volume of water equal to and at the same temperature as the fluidextract was taken as the standard.

4. Preparation of Fluidextract of Belladonna Root by Percolation - For the purpose of comparing regular U.S.P. XI fluidextracts with those made by different repercolation processes or other methods, two fluidextracts of belladonna root were made in accordance with the directions given in the U.S.P. XI monograph. The factors taken into consideration as a basis of comparison were: total alkaloids, total extractive, specific gravity, amount of menstruum used, the time required in the manufacture and the average temperature of the extraction.

Two 1000-Gm. portions of belladonna root were used and each portion was moistened with 600 cc. of menstruum. Since it was found that packing from the top increased the efficiency of extraction (90), the drug was packed in the percolators from the top to a volume of 2675 cc. The drug

was added in about five portions with agitation after each addition in order to remove air spaces. Then pressure was gradually applied from the top until no further decrease in volume was observed. The dimensions of the percolators were as follows: length 56.5 cm., internal diameter at the top 11.5 cm. After maceration for 48 hours the percolate was allowed to flow at the rate of 2 cc. per minute, a reserve portion of 800 cc. and 2200 cc. of weak percolate being collected in each case. At this point an appropriate test for alkaloids was made by use of Mayer's reagent after evaporating 5 cc. portions of the percolate to dryness and acidifying with dilute sulphuric acid. The results of the test were negative in each case.

The weak percolate was placed in an appropriate vacuum distillation apparatus and distilled at a temperature not exceeding 60 degrees centigrade. With sample A vacuum distillation was continued until bubbles no longer were formed on the surface of the extract; with sample B vacuum distillation was continued until no more distillate came through the condenser. As a result of the difference in the vacuum distillation the residue from the weak percolate of sample A was a soft extract that dissolved readily in the warmed reserve portion while that from sample B was a much firmer extract that dissolved with difficulty in the warmed reserve portion. Fluidextract B precipitated much more than fluidextract A after storing one week.

The experimental details and results are listed in the following table:

Table I

Preparation of Fluidextract of Belladonna Root by U.S.P. XI Percolation.

	Fldext. A	Fldext. B	Average
Average Temperature During Experiment	25°C.	25°C.	25°C.
Menstruum Used in cc.	5100	5100	5100
Volume of Drug in cc.	2675	2675	2675
Length of Drug Column in cm.	37	37	37
Time of Operator Required in Hrs.	4.2	4.2	4.2
Total Elapsed Time Required in Hrs.	108.5	108.5	108.5
Gm. Alkaloids in 1000 cc.	5.9	5.7	5.8
Gm. Extractive Matter in 1000 cc.	168.4	172.5	170.4
Specific Gravity	0.979	0.982	0.980

5. Preparation of Fluidextract of Belladonna Root by U.S.P. XI Repercolation - The purpose of the experiment was to compare fluidextract of belladonna root made by the U.S.P. XI repercolation process using different methods of packing with the fluidextract of belladonna root made by the regular percolation process, noting the amount of menstruum required and the time required in each case.

The two methods of packing used were "from the top" and "in sections". In the method of packing designated as "from the top", the moistened drug was introduced into the percolator in small portions with slight agitation of the percolator to promote even distribution, and after all the

drug had been thus introduced it was packed down from the top, using a wooden potato masher and starting with light pressure which was gradually increased. In the method of packing designated as "in sections", the drug was introduced in about eight portions and each separate portion packed down. The method of packing in sections gave tighter packing as evidenced by the smaller volume of packed drug.

The amount of menstruum used in the moistening was 60 cc. per 100 Gm. of drug, and the rate of flow was 2 cc. a minute as in the previous experiment. The U.S.P. XI repercolation process was followed using one set of percolators with each method of packing. However, instead of collecting the last reserve of 500 cc. in one portion as directed by the U.S.P. XI, successive portions of 300 and 200 cc. respectively were collected.

In order to check on the extraction, each portion was analyzed for alkaloids and extractive matter. By using part of each reserve portion a sample of finished fluidextract was prepared and analyzed for alkaloids and extractive matter. The analytical results for the finished fluidextract served as a check on the analyses of the individual reserve portions.

A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive matter are shown in Table II.

Table II

Preparation of Fluidextract of Belladonna Root by U.S.P. XI Repercolation.

A. Experimental Details.

	Packed from Top.	Packed in Sections.
Dimensions of the Percolators.		
For 500 Gm. Portion		
Length	42.5 cm.	36.5 cm.
Width	8.5 cm.	7.25 cm.
For 300 Gm. Portion		
Length	36.5 cm.	29.5 cm.
Width	7.5 cm.	5.75 cm.
For 200 Gm. Portion		
Length	28.5 cm.	27.5 cm.
Width	5.5 cm.	5.75 cm.
Volume of Packed Drug.		
500 Gm. Portion	1325 cc.	1125 cc.
300 Gm. Portion	750 cc.	675 cc.
200 Gm. Portion	530 cc.	450 cc.
Total	2605 cc.	2250 cc.
Length of Drug Column.		
500 Gm. Portion	26 cm.	27 cm.
300 Gm. Portion	17 cm.	22.5 cm.
200 Gm. Portion	21 cm.	17.5 cm.
Total	64 cm.	67.0 cm.
Average Temperature During Experiment	25°C.	25°C.
Menstruum Used in cc.	2500	2075

(Table continued on next page.)

	Packed from Top.	Packed in Sections.
Time of Operator Required in Hrs.	2.2	2.9
Total Elapsed Time in Hours	176	176
Amount of Weak Percolate in cc. Collected at the End	150	250

B. Assay Results.

Gm. Alkaloids in Various Fractions of Percolate.		
First Reserve-200 cc.	1.3	1.5
Second Reserve-300 cc.	2.4	2.6
Third Reserve		
First Portion-300 cc.	1.5	1.3
Second Portion-200 cc.	0.1	0.1
Total 1000 cc.	5.3	5.5
Mixed Fluidextract Calculated on 1000 cc.	5.2	5.5
Weak Percolate Collected at End	0.1	0.2
Gm. Extractive Matter in Various Fractions of Percolate.		
First Reserve-200 cc.	20.1	19.8
Second Reserve-300 cc.	39.3	40.1
Third Reserve		
First Portion-300 cc.	42.3	44.4
Second Portion-200 cc.	18.9	20.4
Total 1000 cc.	120.6	124.7
Weak Percolate Collected at End	14.2	19.2

(Table continued on next page.)

	Packed from Top.	Packed in Sections.
Mixed Fluidextract Calculated on 1000 cc.	119.4	122.1
Specific Gravity of Mixed Fluidextract	0.957	0.959

The data in the preceding table indicate that the fluidextract prepared from the drug packed in sections contained slightly more alkaloids and extractive matter than the fluidextract prepared from the drug packed from the top. Packing from the top gave a larger volume of packed drug than packing in sections, but in the latter case the length of the drug column was greater because smaller percolators were used.

It seemed desirable to carry out another similar experiment in which the two different methods of packing would be compared using corresponding percolators of the same size. Under these conditions the greater drug volume obtained in packing from the top gives a longer drug column than is obtained when packing in sections. Accordingly, an experiment was carried out using corresponding percolators of the same size for each method of packing, but keeping all other details of the experiment the same as in the preceding experiment. Experimental details and analytical data are given in Table III.

Table III

Preparation of Fluidextract of Belladonna Root by U.S.P. XI Repercolation.

A. Experimental Details.

	Packed from Top.	Packed in Sections.
Dimensions of the Percolators.		
For 500 Gm. Portion		
Length	42 cm.	43 cm.
Width	8.5 cm.	8.7 cm.
For 300 Gm. Portion		
Length	37 cm.	36.3 cm.
Width	7.9 cm.	7.5 cm.
For 200 Gm. Portion		
Length	29 cm.	29 cm.
Width	5.8 cm.	5.5 cm.
Volume of Packed Drug.		
500 Gm. Portion	1300 cc.	1100 cc.
300 Gm. Portion	750 cc.	650 cc.
200 Gm. Portion	475 cc.	430 cc.
Total	2525 cc.	2180 cc.
Length of Drug Column.		
500 Gm. Portion	27.5 cm.	25 cm.
300 Gm. Portion	18.5 cm.	16.5 cm.
200 Gm. Portion	19.0 cm.	18.5 cm.
Total	65.0 cm.	60.0 cm.
Average Temperature During Experiment	23.5°C.	23.5°C.
Menstruum Used in cc.	2700	2545

(Table continued on next page.)

	Packed from Top.	Packed in Sections.
Time of Operator Required in Hrs.	2.1	2.7
Total Elapsed Time Required in Hrs.	176	176
Amount of Weak Percolate in cc. Collected at End	180	210

B. Assay Results.

Gm. Alkaloids in Various Fractions of Percolate.		
First Reserve-200 cc.	1.6	1.5
Second Reserve-300 cc.	2.6	2.5
Third Reserve		
First Portion-300 cc.	1.6	1.8
Second Portion-200 cc.	0.1	0.1
Total 1000 cc.	5.9	5.9
Mixed Fluidextract Calculated on 1000 cc.	5.9	6.1
Weak Percolate Collected at End	0.1	0.1
Gm. Extractive Matter In Various Fractions of Percolate.		
First Reserve-200 cc.	19.0	20.2
Second Reserve-300 cc.	37.4	37.4
Third Reserve		
First Portion-300 cc.	39.3	38.4
Second Portion-200 cc.	21.3	19.4
Total 1000 cc.	117.0	115.4

(Table continued on next page.)

	Packed from Top.	Packed in Sections.
Weak Percolates Collected at End	13.2	15.2
Mixed Fluidextract Calculated on 1000 cc.	117.4	116.2
Specific Gravity of Mixed Fluidextract	0.956	0.954

The data in the preceding table indicate that the fluidextracts made by the two different methods of packing in corresponding percolators of the same size are practically identical. Hence there seems to be no particular advantage in either method of packing in repercolation of belladonna root.

6. Preparation of Fluidextract of Belladonna Root by N.F. II Repercolation - An experiment was carried out in order to compare the fluidextract of belladonna root made by the N.F. II repercolation process using different methods of packing, with the fluidextract of belladonna root made by the U.S.P. XI repercolation process.

The N.F. II repercolation process differs from the U.S.P. XI repercolation process in that the drug and respective reserved portions of percolate are divided into portions of 500, 325 and 175 Gm. or cc. as compared with 500, 300 and 200 Gm. or cc. in the U.S.P. XI process. The notable difference between the two processes is that from the second portion of 325 Gm. of drug in the N.F. II repercolation process only 650 cc. of weak percolate is collected against 1000 cc. collected in five 200 cc. portions in the U.S.P. XI repercolation process.

The N.F. II repercolation process (91) is as follows:

"Take of the drug, in powder of the prescribed fineness, one thousand (1000) grams, and divide it into three portions, of five hundred (500), three hundred and twenty-five (325), and one hundred and seventy-five (175) grams, respectively.

"Moisten the first portion of the drug (500 Gm.) with the menstruum and percolate in the usual manner. Set aside the first one hundred and seventy-five (175) cubic centimeters of percolate, and continue until fifteen hundred (1500) cubic centimeters more of percolate have passed, which must be received in several portions, so that the more concentrated will be separate from the last, weak percolate.

"Then moisten the second portion of the drug (325 Gm.) with the more concentrated percolates received during the preceding operation after the first one hundred and seventy-five (175) cubic centimeters have passed, and percolate again in the usual manner, using the several reserved percolates, successively, as menstrua. Set aside the first three hundred and twenty-five (325) cubic centimeters and continue the percolation until six hundred and fifty (650) cubic centimeters more have passed, which should also be received in several portions.

"Finally moisten the third portion of the drug (175 Gm.) with the most concentrated of the last reserved percolates, and proceed as directed for the second portion. Collect five hundred (500) cubic centimeters of percolate and mix with the two portions (three hundred and twenty-five (325) and one hundred and seventy-five (175) cubic centimeters) previously set aside, so as to make one thousand (1000) cubic centimeters of fluidextract.

"Note - If this method is applied to drugs for which the Process B is directed, use a sufficient quantity of menstruum I to obtain the required quantities of percolate, and omit the use of Menstruum II."

Two 1000-Gm. portions of drug were used with the two methods of packing previously described. As in the previous experiments the amount of menstruum used in the moistening was 60 cc. per 100 Gm. drug and the rate of flow was 2 cc. a minute.

In order to check on the extraction, each portion was analyzed for alkaloids and extractive matter. By using part of each reserve portion a sample of finished fluidextract was prepared and analyzed for alkaloids and extractive matter. The analytical results for the finished fluidextract served as a check on the analyses of the individual reserve portions.

A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive matter are shown in Table IV.

Table IV

Preparation of Fluidextract of Belladonna Root by N.F. II Repercolation.

A. Experimental Details.

	Packed from Top.	Packed in Sections.
Dimensions of the Percolators.		
For 500 Gm. Portion		
Length	42.5 cm.	36.5 cm.
Width	8.5 cm.	7.25 cm.

(Table continued on next page.)

	Packed from Top.	Packed in Sections.
For 325 Gm. Portion		
Length	36.5 cm.	29.5 cm.
Width	7.5 cm.	5.75 cm.
For 175 Gm. Portion		
Length	28.5 cm.	27.5 cm.
Width	5.5 cm.	5.75 cm.
Volume of Packed Drug.		
500 Gm. Portion	1300 cc.	1125 cc.
325 Gm. Portion	875 cc.	750 cc.
175 Gm. Portion	465 cc.	385 cc.
Total	2640 cc.	2260 cc.
Length of Drug Column.		
500 Gm. Portion	27.5 cm.	29 cm.
325 Gm. Portion	22.0 cm.	27.5 cm.
175 Gm. Portion	20.0 cm.	17.5 cm.
Total	69.5 cm.	74.0 cm.
Average Temperature During Experiment	24.5°C.	24.5°C.
Menstruum Used in cc.	2480 cc.	2480 cc.
Time of Operator Required in Hrs.	1.6	2
Total Elapsed Time Required in Hrs.	176	176

B. Assay Results.

Gm. Alkaloids in Various Fractions of Percolate.		
First Reserve-175 cc.	1.3	1.3

(Table continued on next page.)

	Packed from Top.	Packed in Sections.
Second Reserve-325 cc.	2.4	2.9
Third Reserve		
First Portion-300 cc.	1.5	1.3
Second Portion-200 cc.	0.2	0.2
Total 1000 cc.	5.4	5.7
Mixed Fluidextract Calculated on 1000 cc.	5.5	5.6
Gm. Extractive Matter in Various Fractions of Percolate.		
First Reserve-175 cc.	19.0	19.7
Second Reserve-325 cc.	44.6	44.5
Third Reserve		
First Portion-300 cc.	43.7	42.0
Second Portion-200 cc.	19.1	19.4
Total 1000 cc.	126.4	125.6
Mixed Fluidextract Calculated on 1000 cc.	123.9	122.9
Specific Gravity of Mixed Fluidextract	0.956	0.956

It was demonstrated that in the manufacture of fluidextract of belladonna root by the use of the U.S.P. XI repercolation process there was always a considerable portion of weak percolate left after the collection of the last reserve portion of 500 cc. The N.F. II repercolation process, however, required a little more fresh menstruum at this point in order to obtain the 500 cc. reserve portion. The U.S.P. X repercolation process collected 800 cc. in 200 cc. portions at this point which gave an excess of about 55 cc. of weak percolate in the manufacture of fluidextract of belladonna root and about 40 cc. of weak percolate in the manu-

facture of fluidextracts of cinchona and nux vomica. These details were recorded, but not published in a previous investigation (39).

Within experimental error, the N.F. II repercolation process proved to be as efficient as the U.S.P. XI repercolation process in the manufacture of fluidextract of belladonna root. The averages of the results of both processes are compared in Table V.

Table V

Gm. Alkaloids in 1000 cc. Fluidextract Belladonna Root.

	Packed from Top.	Packed in Sections.
U.S.P. XI Repercolation	5.6	5.8
N.F. II Repercolation	5.5	5.7

7. A Study of the Efficiency of Extraction Using Various Forms of Percolators - The purpose of this experiment was to compare the efficiency of the forms of percolators used in drug extraction and also to compare the results using belladonna root with the recently published results of J. Büchi and K. Feinstein (41) using cinchona.

Two percolators were used of each of the following types:

(a) Ordinary Oldberg Percolator
Length of Column 27.3 cm.
Internal Diameter at Top 5.7 cm.

(b) Funnel
Length of Column 15.5 cm.
Internal Diameter at Top 17.5 cm.

(c) Pyrex Glass Tube
Length of Column 90 cm.
Internal Diameter at Top 2.5 cm.

(d) Pyrex Glass Tube
Length of Column 64 cm.
Internal Diameter at Top 4 cm.

The glass tubes were fitted with one-holed rubber stoppers that were channelled so as to allow suitable drainage. All the percolators were provided with glass tubes, rubber tubing and pinch clamps and all were graduated to about four-fifths their capacity. The glass tubes of 2.5 cm. diameter had the lowest capacity; each of these tubes had a capacity of 150 Gm. of moistened drug, hence this quantity was used in all of the percolators. The quantity of moistening liquid used was 25 cc. per 100 Gm. of drug, since it was found by Husa and Yates (92) that this proportion of moistening liquid gave more efficient extraction than larger proportions.

The best rate of flow for most efficient extraction was found by Husa and Huyck (93) to be 2 to 3 cc. a minute for 800 Gm. of powdered belladonna root. The rate of flow in this experiment was regulated in proportion to the quantity of drug used, namely 0.4 cc. a minute. From previous work Husa and Huyck (90) found the quickest extraction of belladonna root to take place when the drug was placed in the percolator in portions with constant agitation and packed from the top. Therefore, this method of packing was adopted in this experiment. From preliminary experiments with the narrow tube, it was found that if the drug was packed tightly from the top about fifteen hours were required for the liquid to reach the bottom of the percolator, and the rate of free flow averaged about 0.079 cc. a minute for the first four hours. To increase the rate of flow, the narrowest tubes were packed more lightly in this experiment so as to allow a free flow of at least 0.4 cc. a minute. The volume of the packed drug was found to be about 410 cc. In the wider tubes the

drug was packed to a volume of 400 cc., in the percolator to 375 cc. and in the funnels to 370 cc. The funnels and Oldberg percolators were packed tightly from the top. After the liquid had begun to drop from the lower orifice each portion of drug was allowed to macerate for twenty and one-half hours. This length of maceration after packing was adopted mainly for convenience, because it has been found by Husa and Huyck (93) that maceration for 48 hours after packing only slightly increased the efficiency of extraction of alkaloids and total extractive. As in the U.S.P. XI directions for regular percolation an eighty per cent reserve was collected and in order to study the rate of extraction this reserve was collected in two portions of 60 cc. each. In addition a 60 cc. portion of weak percolate was collected. The room temperature was about 24° C. during the experiment. The experimental details are shown in Table VI. The values for the total quantity of menstruum absorbed by the drug were obtained by subtracting the total amount of percolate collected from the total amount of menstruum which passed below the upper surface of the drug.

Table VI

A Study of the Efficiency of Extraction Using Various Forms of Percolators.

A. Experimental Details.

	2.5 cm. Tube	4 cm. Tube	Funnel	Percolator
Length of Drug Column in cm.	72	30	9.5	17.3
	72	30	9.5	17.3

(Table continued on next page.)

	2.5 cm. Tube	4 cm. Tube	Funnel	Percolator
Time Required for Liquid to Reach Lower Orifice in Min.	228	114	80	85
	213	115	71	96
Quantity of Menstruum Absorbed in cc.	226	220	294	225
	230	217	290	229
Quantity of Menstruum Used in cc.	406	400	474	405
	410	397	470	409
	B. Assay Results.			
Gm. Alkaloids.				
Reserve I	0.51	0.48	0.58	0.61
	0.59	0.47	0.58	0.59
Average	0.55	0.48	0.58	0.60
Reserve II	0.10	0.15	0.17	0.16
	0.10	0.14	0.17	0.13
Average	0.10	0.15	0.17	0.15
Weak Percolate	0.03	0.03	0.04	0.02
	0.03	0.08	0.04	0.03
Average	0.03	0.08	0.04	0.03
Total of Reserves and Weak Percolate	0.64	0.71	0.79	0.79
	0.72	0.69	0.79	0.75
Average	0.68	0.70	0.79	0.77

(Table continued on next page.)

	2.5 cm. Tube	4 cm. Tube	Funnel	Percolator
Gm. Extractive Matter.				
Reserve I	9.37	8.87	8.13	7.97
	8.96	7.80	7.90	7.60
Average	9.17	8.34	8.02	7.79
Reserve II	7.87	7.96	8.13	7.96
	8.05	8.11	7.78	7.63
Average	7.96	8.04	7.96	7.80
Weak Percolate	6.84	7.34	5.97	6.61
	6.06	6.32	6.11	7.73
Average	6.45	6.83	6.04	7.17
Total of Reserves and Weak Percolate	24.08	24.17	22.23	22.54
	23.07	22.23	21.79	22.96
Average	23.58	23.20	22.01	22.75

From the table of summarized duplicate experiments the percolator and funnel show more efficient extraction of alkaloids than either of the tubes in the two reserve portions as well as in the totals. The funnel and the percolator appear equally efficient in the extraction of the alkaloids. On the contrary the tubes show a little more efficient extraction of extractive matter in the reserves as well as in the totals, the narrow tube giving the greatest yield. In this tube the increase of yield is mainly due to the high content of extractive matter in the first reserve. Although the percolator and the funnel produced nearly the same amount of total extractive, the funnel seemed to show a sudden decrease

of extractive matter in the weak percolate portion. For this reason the percolator may be given a little preference over the funnel.

J. Buchi and K. Feinstein (41) carried out a similar experiment on cinchona; the amount of moistening menstruum used was 40 cc. per 100 Gm. of cinchona as compared with 25 cc. per 100 Gm. of belladonna root. On the basis of content of alkaloids in the reserve portions, the 2.5 cm. tube was most efficient for cinchona while the funnel was most efficient for belladonna root. On the basis of content of extractive matter in the reserve portions the 2.5 cm. tube was most efficient for both cinchona and belladonna root. Therefore, with both cinchona and belladonna root the longer drug columns slightly increased the yields of extractive matter. In conclusion, the results with belladonna root are in general agreement with the results recently published on cinchona in that the form of percolator does not seem to make any great difference in the percentage of extraction of alkaloids or extractive matter.

J. U. Lloyd (12) measured the effect of varying the diameter and heights of tubes in the percolation of cimicifuga. When maceration after packing was omitted a tenfold increase in length of the tubes doubled the yield of extractive matter. Also when maceration after packing was employed an increase in the length of the tube produced a greater increase in yield of extractive matter than the same increase in length produced with cinchona or belladonna root. From the results it is concluded that with cimicifuga the increased length of drug column increased the yield of extractive matter more than with either belladonna root or cinchona.

In previous unpublished work by the author (94) a comparison of the

efficiency of the Oldberg type percolator with the diacolator type of nearly the same diameter was made. The experimental details and the results of the experiment were as follows:

"Using powdered belladonna root, percolations were carried out to compare the efficiency of the Oldberg type of percolator with the straight glass tubes of uniform diameter used in diacolation. The Oldberg percolator used was the quart size having a length of 45.5 cm. and an internal diameter at the top of 7.5 cm. The diacolator was a straight glass tube 70.5 cm. long and having an internal diameter of 6.5 cm. Five hundred gram portions of belladonna root in No. 40 powder were used. Each portion was moistened with 300 cc. of the menstruum (5 volumes alcohol - 1 volume water). In packing 2.5 Gm. of glass wool was placed in the bottom of the percolator and diacolator, respectively. After packing in the percolator and diacolator the volume of the packed drug was approximately 1075 cc. in each case. The height of the column of drug was 23 cm. in the Oldberg percolator and 28.5 cm. in the diacolator. Percolation was commenced without allowing time for maceration either before or after packing. In each case a first percolate of 250 cc. was collected in 23 hours and a second percolate of 250 cc. was likewise collected in 23 hours. The temperature was approximately 25° C. during the percolation.

Gm. Alkaloids.

	Oldberg Percolator.	Diacolator.
First Percolate of 250 cc.	1.4	1.4
Second Percolate of 250 cc.	0.8	0.7
Total	2.2	2.1

Gm. Total Extractive.

First Percolate of 250 cc.	28.9	29.0
Second Percolate of 250 cc.	24.0	26.0
Total	52.9	55.0

The above results do not show any appreciable advantage for either the Oldberg type percolator or the diacolator." These results are in general agreement with the results of this experiment.

8. Analysis of the Repercolation Process - Since it has been stated by Bennett and Cocking (36) that a weak solution of the extractive of a drug is usually a better solvent for the active constituents than the original menstruum, an experiment was carried out in order to throw some light on the value of this statement as applied to the extraction of belladonna root.

In U.S.P. repercolation, 1000 Gm. of drug is divided into three portions of 500 Gm., 300 Gm. and 200 Gm. respectively, which are successively percolated. In the present experiment the quantity of the first portion was doubled. From the 1000-Gm. portion of drug a reserve percolate of 400 cc. and five successive portions of weak percolate of 600 cc. each were collected. Each portion of weak percolate was thoroughly mixed and divided into two equal parts, one part being set aside for analysis and

the other part being used in percolating another portion of drug weighing 300 Gm. From this portion of drug a reserve percolate of 300 cc. and five portions of weak percolate of 200 cc. each were collected. One-half of each portion of weak percolate was set aside for analysis and the other half used in percolating another portion of drug. The third portion of drug weighed 100 Gm., as compared with the 200 Gm. specified in the U.S.P. repercolation. Obviously it was necessary to reduce the last portion of drug by one-half since half of each of the weak percolates to be used had been reserved for analysis.

By following the procedure outlined in the preceding paragraph it was possible to conduct repercolation according to the U.S.P. directions, with the exception that the first portion of drug was doubled and the third portion reduced by one-half in order to have a sufficient quantity of each portion of weak percolate for analysis. In addition to this, a further percolation was carried out using a 300-Gm. portion of drug which was percolated with fresh menstruum instead of with weak percolate, a reserve percolate of 300 cc. and five successive 200-cc. portions of weak percolate being collected. By analysing these percolates and comparing them with the percolates obtained from a 300-Gm. portion of drug percolated with weak percolate, it became possible to draw a conclusion as to the value of the dissolved constituents of the drug present in the weak percolates.

In order to check on the extraction, each portion was analyzed for alkaloids and extractive matter. By using part of each reserve portion a sample of finished fluidextract was prepared and analyzed for alkaloids

and extractive matter. The analytical results of the finished fluidextract served as a check on the analysis of the individual reserve portions.

A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive matter are shown in Table VII.

Each portion of drug was moistened, allowed to macerate for 15 minutes and packed from the top. The moistening liquid was used in the proportion of 25 cc. of liquid to 100 Gm. of drug. Each percolation was conducted in duplicate.

Table VII

Analysis of the Repercolation Process.

A. Experimental Details.

Number	A-1	A-2	B-1*	B-2*	C-1	C-2	D-1**	D-2**
Wt. of Drug in Gm.	1000	1000	300	300	100	100	300	300
Volume of Packed Drug.	2450	2450	745	745	250	250	745	745
Time Required for Liquid to Reach Lower Orifice in Min.	270	270	150	120	105	105	105	120
Time of Maceration after Packing in Minutes	510	510	750	750	570	570	750	750
Rate of Flow in cc. per Min.	3.5	3.5	1.8	1.8	1.3	1.3	2.5	2.5
Menstruum Absorbed in cc.	1465	1510	550	500	150	150	575	575
Fresh Menstruum Used in cc.	4865	4910	1850	1800	500	500	1875	1875

* Percolated with weak percolate.

** Percolated with fresh menstruum.

B. Assay Results.

A. Percolates from 1000-Gm. Portions of Drug.

	Gm. Alkaloids in Fractions of Percolate.		
	A-1	A-2	Average of A-1 and A-2.
Reserve-400 cc.	3.94	4.69	4.31
Weak Percolate-600 cc.	0.88	0.74	0.81
Weak Percolate-600 cc.	0.24	0.28	0.26
Weak Percolate-600 cc.	0.28	0.12	0.20
Weak Percolate-600 cc.	0.26	0.12	0.19
Weak Percolate-600 cc.	0.36	0.10	0.23
Total	5.96	6.05	6.00

B. Percolates from 300-Gm. Portions of Drug Percolated with Weak Percolate.

	Gm. Alkaloids in Fractions of Percolate.		
	B-1	B-2	Average of B-1 and B-2.
Reserve-300 cc.	2.01	1.83	1.92
Weak Percolate-200 cc.	0.20	0.20	0.20
Weak Percolate-200 cc.	0.10	0.18	0.14
Weak Percolate-200 cc.	0.08	0.08	0.08
Weak Percolate-200 cc.	0.06	0.08	0.07
Weak Percolate-200 cc.	0.04	0.04	0.04
Total	2.49	2.41	2.45

C. Percolates from 100-Gm. Portions of Drug.

	Gm. Alkaloids in Fractions of Percolate.		
	C-1	C-2	Average of C-1 and C-2.
Reserve-250 cc.	0.79	0.83	0.81
Residual Percolate of 100 cc.	0.02	0.02	0.02
Total	0.81	0.85	0.83

D. Percolates from 300-Gm. Portions of Drug Percolated with Fresh Menstruum.

	Gm. Alkaloids in Fractions of Percolate.		
	D-1	D-2	Average of D-1 and D-2.
Reserve-300 cc.	1.76	1.87	1.81
Weak Percolate-200 cc.	0.11	0.09	0.10
Weak Percolate-200 cc.	0.06	0.04	0.05
Weak Percolate-200 cc.	0.03	0.03	0.03
Weak Percolate-200 cc.	0.03	0.03	0.03
Weak Percolate-200 cc.	0.04	0.03	0.03
Total	2.03	2.09	2.05
Mixed Fluidextract Calculated on 1000 cc.	5.2	5.5	5.4

A. Percolates from 1000-Gm. Portions of Drug.

	Gm. Extractive Matter in Fractions of Percolate.		
	A-1	A-2	Average of A-1 and A-2.
Reserve-400 cc.	66.5	61.3	63.9

(Table continued on next page.)

	A-1	A-2	Average of A-1 and A-2.
Weak Percolate-600 cc.	75.7	71.0	73.4
Weak Percolate-600 cc.	44.8	47.9	46.4
Weak Percolate-600 cc.	21.1	21.1	21.1
Weak Percolate-600 cc.	6.2	6.2	6.2
Weak Percolate-600 cc.	3.5	3.5	3.5
Total	217.8	211.0	214.5

B. Percolates from 300-Gm. Portions of Drug Percolated with Weak Percolate.

Gm. Extractive Matter in Fractions of Percolate.

	B-1	B-2	Average of B-1 and B-2.
Reserve-300 cc.	52.6	50.6	51.8
Weak Percolate-200 cc.	23.1	23.7	23.4
Weak Percolate-200 cc.	17.0	18.2	17.6
Weak Percolate-200 cc.	13.6	13.5	13.6
Weak Percolate-200 cc.	9.8	10.6	10.2
Weak Percolate-200 cc.	6.6	7.1	6.9
Total	122.7	124.0	122.5

C. Percolates from 100-Gm. Portions of Drug.

Gm. Extractive Matter in Fractions of Percolate.

	C-1	C-2	Average of C-1 and C-2.
Reserve-250 cc.	37.0	37.3	37.2

(Table continued on next page.)

	C-1	C-2	Average of C-1 and C-2.
Residual-100 cc.	8.9	8.9	8.9
Total	45.9	46.2	46.1

D. Percolates from 300-Gm. Portions of Drug Percolated with Fresh Menstruum.

	Gm. Extractive Matter in Fractions of Percolate.		
	D-1	D-2	Average of D-1 and D-2.
Reserve-300 cc.	41.6	39.8	40.7
Weak Percolate-200 cc.	16.5	14.3	15.4
Weak Percolate-200 cc.	6.7	5.6	6.2
Weak Percolate-200 cc.	2.3	2.3	2.3
Weak Percolate-200 cc.	1.3	1.3	1.3
Weak Percolate-200 cc.	1.0	1.0	1.0
Total	69.4	64.3	66.9
Mixed Fluidextract Calculated on 1000 cc.	148.0	150.0	149.0
Specific Gravity of Mixed Fluidextract	0.957	0.957	0.957

Because it has been previously shown by Husa and Magid (95) that the alkaloidal yield of belladonna root was greater with alcohol-water menstruum than with chloroform-ether solvent used in the official assay, the amount of alkaloids obtained in the percolation of the drug with fresh menstruum was taken as the alkaloidal content of the drug. In considering the extraction of the 300-Gm. portions of drug with fresh menstruum and with weak percolate, the slower rate of flow employed in repercola-

tion is pointed out. From the results of the analyses of the percolates from the 300-Gm. portions of drug percolated with weak percolate 2.45 Gm. of alkaloids was extracted of the total of 2.90 Gm. of alkaloids present (2.05 Gm. of alkaloids in the 300-Gm. portion of drug and 0.85 Gm. of alkaloids in the weak percolate). When percolating with weak percolate there was 0.40 Gm. more of alkaloids present in the percolates obtained than when fresh menstruum was used. However, there was 0.45 Gm. less than the total quantity of alkaloids present in the weak percolate and in the drug. The averages of the results of the extraction of duplicate 300-Gm. portions using fresh menstruum and weak percolate are compared in Table VIII.

Table VIII

Comparison of the Extraction of 300-Gm. Portions of Belladonna Root with Fresh Menstruum and Weak Percolate.

Gm. Alkaloids in the Various Fractions of Percolate.	Fresh Menstruum.	Weak Percolate.
Reserve-300 cc.	1.81	1.92
Weak Percolate-200 cc.	0.10	0.20
Weak Percolate-200 cc.	0.05	0.14
Weak Percolate-200 cc.	0.03	0.08
Weak Percolate-200 cc.	0.03	0.07
Weak Percolate-200 cc.	0.03	0.04
Total	2.05	2.45

(Table continued on next page.)

Gm. Extractive Matter in Various Fractions of Percolate.	Fresh Menstruum.	Weak Percolate.
Reserve-300 cc.	40.7	51.8
Weak Percolate-200 cc.	15.4	23.4
Weak Percolate-200 cc.	6.2	17.6
Weak Percolate-200 cc.	2.3	13.6
Weak Percolate-200 cc.	1.3	10.2
Weak Percolate-200 cc.	1.0	6.9
Total	66.9	122.5

Table VIII shows that in the extraction of the 300-Gm. portion with weak percolate in U.S.P. XI repercolation, the last 200-cc. portion of weak percolate collected contained only 0.04 Gm. alkaloids and comparatively little extractive matter. With fresh menstruum the alkaloids present were rapidly extracted. Of the 2.90 Gm. of alkaloids present in the drug and weak percolates in the extraction of the 300-Gm. portions with weak percolate about 65 per cent was extracted in the reserve portion. In the weak percolates there was a gradual decrease in alkaloidal content. The fifth percolate in each case showed that only small quantities of alkaloids were present. The results of this experiment show that a weak solution of extractive of belladonna root is not a better solvent for the active principles of the drug than the original menstruum.

The amounts of alkaloids and extractive matter in 1000 cc. of finished fluidextract was taken as 100 per cent in calculating the percentage of extraction of alkaloids and extractive matter in the various fractions collected throughout the repercolation process. These amounts,

which were calculated from the amounts of alkaloids and extractive matter in the three reserve portions, were found to be 5.7 Gm. of alkaloids and 158.2 Gm. of extractive matter in 1000 cc. of mixed fluidextract. The percentages of alkaloids and extractive matter in the various fractions collected are shown in Table IX.

Table IX

Percentage Extraction of Alkaloids and Extractive Matter in the Various Portions Collected in the U.S.P. XI Repercolation of Belladonna Root.

First Portion-500 Gm.	Per Cent Alkaloids.	Per Cent Extractive Matter.
Reserve-200 cc.	37.9	20.2
Weak Percolate-300 cc.	7.2	23.2
Weak Percolate-300 cc.	2.3	14.7
Weak Percolate-300 cc.	1.8	6.8
Weak Percolate-300 cc.	1.8	2.0
Weak Percolate-300 cc.	2.1	1.1
Second Portion-300 Gm.		
Reserve-300 cc.	33.7	32.7
Weak Percolate-200 cc.	3.5	14.8
Weak Percolate-200 cc.	2.5	11.1
Weak Percolate-200 cc.	1.4	8.6
Weak Percolate-200 cc.	1.2	6.4
Weak Percolate-200 cc.	0.7	4.4
Third Portion-200 Gm.		
Reserve-500 cc.	28.4	47.0

9. Preparation of Fluidextract of Belladonna Root by a Modified Repetition Diacolation Process. In 1930 repetition diacolation was described by H. Breddin (43) and it is taken up under "New Drug Extraction Processes" in the preceding historical review. In applying this process to the extraction of belladonna root, it was obvious that sufficient menstruum was not used in repetition diacolation to exhaust the drug. Therefore, the process was modified by collecting twice as much weak percolate as directed in repetition diacolation. Furthermore, the menstruum remaining in the drug near the end of the percolation of each portion of drug was not displaced by water as was done by Breddin.

As directed in repetition diacolation heavy walled glass tubes were used as percolators. They were of the following dimensions: length 65 cm., thickness of wall 5 mm., internal diameter 4.1 cm., external diameter 5.1 cm. The glass tubes were fitted with one-holed rubber stoppers which were channelled, so as to allow suitable drainage. Also they were provided with glass tubing, rubber tubing and pinch clamps and were graduated to show the volume of drug. The glass tubes had a capacity of 250 Gm. of moistened drug.

The process, which was carried out in duplicate, was as follows. Divide 750 Gm. of moderately coarsely powdered belladonna root into three equal portions, consisting of 250 Gm. each. Mix the first portion with 62.5 cc. of the menstruum to render it evenly and distinctly damp, transfer the dampened powder to the percolator and allow it to stand for about fifteen minutes. Then pack the drug in the percolator from the top, saturate it with menstruum, and allow it to macerate for 48 hours. Then

proceed with the percolation, first collecting and reserving 125 cc. of percolate, and afterward collecting five successive portions of 150 cc. each, numbering them in the order in which they are obtained. Dampen the second portion of 250 Gm. of drug with 62.5 cc. of the first of the 150-cc. portions of percolate from the preceding lot of drug, and carry out the percolation as just directed for the first lot, excepting that the five 150-cc. portions of percolate from the first lot of drug shall first be used as menstruum, in the order in which they were received, followed by as much fresh menstruum as needed. Reserve the first 125 cc. of percolate and then collect five successive portions of 150 cc. each, numbering them in the order in which they are collected. Now dampen the third portion of 250 Gm. of the drug with 62.5 cc. of the first numbered portion of percolate from the second lot of drug, and proceed with the percolation as before, using as the menstruum the successive 150-cc. portions of percolate from the second lot of drug in the order received. Collect and reserve 500 cc. of percolate. Mix the three reserved percolates from the three lots of drug to make 750 cc. of fluidextract.

In order to check on the extraction, each portion was analyzed for alkaloids and extractive matter. By using part of each reserve portion a sample of finished fluidextract was prepared and analyzed for alkaloids and extractive matter. The analytical results of the finished fluidextract served as a check on the analyses of the individual reserve portions.

A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive matter are shown

in Table X.

Table X

Preparation of Fluidextract of Belladonna Root by a Modified Repetition Diacolation Process.

A. Experimental Details.

Portion	1-A	1-B	2-A	2-B	3-A	3-B
Volume of Packed Drug in cc.	650	650	650	650	650	650
Height of Drug Column in cm.	47.5	47.5	47.5	47.5	47.5	47.5
Time Required for Liquid to Reach Lower Orifice in min.	450	450	1050	1170	930	1050
Time of Maceration after Packing in hours.	38.5	38	40	37.5	45	43
Rate of Flow in cc. a min.	1.4	1.4	1.3	0.69	1.2	1.2
Menstruum Absorbed by Drug in cc.	307.5	312.5	435	405	440	415
Fresh Menstruum Used in cc.	1250	1250	560	530	190	165

B. Assay Results.

Gm. Alkaloids in Various Fractions of Percolate.	A	B	Average of A and B.
First Reserve-125 cc.	1.4	1.4	1.4
Second Reserve-125 cc.	1.5	1.4	1.5

(Table continued on next page.)

	A	B	Average of A and B.
Third Reserve-500 cc.	1.7	2.1	1.9
Total 750 cc.	4.6	4.9	4.8
Total Calculated on 1000 cc.	6.1	6.5	6.3
Mixed Fluidextract Calculated on 1000 cc.	6.1	6.4	6.3
Gm. Extractive Matter in Various Fractions of Percolate.			
First Reserve-125 cc.	21.6	19.4	20.5
Second Reserve-125 cc.	25.2	24.4	24.8
Third Reserve-500 cc.	65.9	72.4	69.1
Total 750 cc.	112.7	116.2	114.4
Total Calculated on 1000 cc.	150.3	154.9	152.6
Mixed Fluidextract Calculated on 1000 cc.	148.5	152.9	150.7
Specific Gravity of Mixed Fluidextract	0.965	0.966	0.966

In comparing modified repetition diacolation with percolation with fresh menstruum the greater lengths of time required for the weak percolates to reach the lower orifices of the second and third portions of the drug are pointed out. To compare the fluidextracts made by simple percolation, U.S.P. XI repercolation and repetition diacolation the content of alkaloids in these fluidextracts is given in Table XI.

Table XI

Gm. Alkaloids in 1000-cc. Portions of Fluidextracts of Belladonna Root Made by U.S.P. XI Simple Percolation, U.S.P. XI Repercolation and Modified Repetition Diacolation.

U.S.P. XI Simple Percolation	5.8
U.S.P. XI Repercolation	5.7
Modified Repetition Diacolation	6.3

The results indicate that the modified repetition diacolation process was better for the extraction of the alkaloids of belladonna root than either simple percolation or repercolation.

10. Preparation of Fluidextract of Belladonna Root by a Modified Repetition Diacolation Process Using Oldberg Percolators - In a previous experiment it was found that a modified repetition diacolation process gave more favorable results than U.S.P. repercolation. In comparing the two processes, it is seen that there are two main points of difference: (a) the type of percolator used, and (b) the proportions of drug in the various portions. Accordingly, an experiment was carried out using the same proportions of drug and the same scheme of collection of percolates as in modified repetition diacolation, but using ordinary Oldberg percolators in place of the cylindrical glass tubes. This experiment was intended to show whether the more favorable results obtained in modified repetition diacolation were due to the apparatus or to the proportions of drug and percolates.

Since the percolators when two-thirds full were found to hold more drug than the tubes, portions of 333.3 Gm. of drug were used instead of

portions of 250 Gm. as in the previous experiment. The method used was as in the preceding modified repetition diacolation experiment.

As in the previous experiments, in order to check on the extraction, each portion was analyzed for alkaloids and extractive matter. By using part of each reserve portion a sample of finished fluidextract was prepared and analyzed for alkaloids and extractive matter. The analytical results of the finished fluidextract served as a check on the analysis of the individual reserve portions.

A summary of the details of the process and an average of the analyses of the various portions for alkaloids and extractive matter are shown in Table XII.

Table XII

Preparation of Fluidextract of Belladonna Root by a Modified Repetition Diacolation Process Using Oldberg Percolators.

A. Experimental Details.

Portion	1-A	1-B	2-A	2-B	3-A	3-B
Volume of Packed Drug in cc.	800	800	800	800	800	800
Height of Drug Column in cm.	20	21	21.5	20.5	20	20
Time Required for Liquid to Reach Lower Orifice in min.	75	70	300	300	360	345
Time of Maceration after Packing in hours	46.5	46.3	37.5	36.5	36.8	36.5

(Table continued on next page.)

Portion	1-A	1-B	2-A	2-B	3-A	3-B
Rate of Flow in cc. a min.	1.6	1.6	1.2	1.2	1.6	1.6
Menstruum Absorbed by Drug in cc.	448	539	563	563	556	546
Fresh Menstruum Used in cc.	1698	1706	630	630	226	212

B. Assay Results.

Gm. Alkaloids in Various Fractions of Percolate.	A	B	Average of A and B.
First Reserve-167 cc.	1.7	1.7	1.7
Second Reserve-167 cc.	1.9	1.8	1.9
Third Reserve-666 cc.	2.6	2.6	2.6
Total 1000 cc.	6.1	6.1	6.2
Mixed Fluidextract Calculated on 1000 cc.	5.9	5.9	5.9
Gm. Extractive Matter in Various Fractions of Percolate.			
First Reserve-167 cc.	23.7	21.3	22.5
Second Reserve-167 cc.	25.7	26.1	25.9
Third Reserve-666 cc.	99.7	99.7	99.7
Total 1000 cc.	149.1	147.1	148.1
Mixed Fluidextract Calculated on 1000 cc.	149.7	149.2	149.4
Specific Gravity of Mixed Fluidextract	0.959	0.959	0.959

For the purpose of comparison, the results of the preceding experiment were calculated on the basis of 1000 Gm. drug and compared with the

preceding results as shown in Table XIII.

Table XIII

Comparison of Tubes and Oldberg Percolators in Modified Repetition Diacolation.

Gm. Alkaloids in Various Fractions of Percolate.	Tubes	Oldberg Percolators.
First Reserve-167 cc.	1.8	1.7
Second Reserve-167 cc.	2.0	1.9
Third Reserve-666 cc.	2.5	2.6
Total 1000 cc.	6.3	6.2
Gm. Extractive Matter in Various Fractions of Percolate.		
First Reserve-167 cc.	27.4	22.5
Second Reserve-167 cc.	33.2	25.9
Third Reserve-666 cc.	92.0	99.7
Total 1000 cc.	152.6	148.1

The results indicate that in modified repetition diacolation Oldberg percolators were just as efficient as cylindrical glass tubes for the extraction of the alkaloids of belladonna root. The extractive matter was extracted somewhat more rapidly in the cylindrical tubes than in the Oldberg percolators. The more favorable results obtained in modified repetition diacolation are not due to the apparatus, but to the proportions of drug and percolates. Although larger amounts of moistening menstruum were used in simple percolation and U.S.P. XI repercolation, the amounts of alkaloids in fluidextracts made by the various processes are sum-

marized in Table XIV.

Table XIV

Gm. Alkaloids in 1000-cc. Portions of Fluidextract of Belladonna Root Made by U.S.P. XI Simple Percolation, U.S.P. XI Repercolation, Modified Repetition Diacolation and Modified Repetition Diacolation Using Oldberg Percolators.

U.S.P. XI Simple Percolation	5.8
U.S.P. XI Repercolation	5.7
Modified Repetition Diacolation	6.3
Modified Repetition Diacolation using Oldberg Percolators	6.2

11. Direct Comparison of the U.S.P. Repercolation and Modified Repetition Diacolation - Previous experiments indicated that modified repetition diacolation gave better results than U.S.P. repercolation in the preparation of fluidextract of belladonna root. However, it seemed desirable to carry out a further experiment in order to eliminate all possible sources of error. In order to equalize such factors as temperature, etc., a portion of fluidextract of belladonna root was made by each of the processes at the same time in the same room. The drug used was taken from the large shipment and again thoroughly mixed. Percolators were chosen so that each portion of the drug filled each percolator two-thirds to three-fourths full when the drug was packed firmly from the top. The U.S.P. XI repercolation process and the modified repetition diacolation process using Oldberg percolators as previously described were followed. By mixing the reserve portions two 1000-cc. portions of finished fluidextract were

prepared and analyzed for alkaloids and extractive matter.

A summary of the details of the process and the results of the analyses of the fluidextracts is shown in Table XV.

Table XV

Direct Comparison of the U.S.P. Repercolation and Modified Repetition Diacolation.

A. Experimental Details.

	U.S.P. XI Repercolation.			Modified Repetition Diacolation.		
Wt. in Gm. of Portion of Drug.	500	300	200	333.3	333.3	333.3
Volume of Packed Drug in cc.	1250	745	485	820	820	820
Height of Drug Column in cm.	27.5	19.5	19	23	23.5	22.5
Time Required for Liquid to Reach Lower Orifice in min.	135	150	115	112	192	255
Time of Maceration after Packing in Hours.	44.8	45	44.8	44.8	44	43
Rate of Flow in cc. a min.	1.5	1.6	0.9	1.6	1.6	1.6
Menstruum Absorbed by Drug in cc.	800	450	300	459	478	522
Fresh Menstruum Used in cc.	2500	250	----	1126	645	188

(Table continued on next page.)

B. Assay Results.

U.S.P. XI Repercolation.	Modified Repetition Diacolation.
Gm. Alkaloids in 1000 cc. of Fluidextract of Belladonna Root.	
5.9	6.4
5.8	6.3
5.9	6.4
5.9	6.3
Average 5.9	Average 6.4
Gm. Extractive Matter in 1000 cc. Fluidextract of Belladonna Root.	
148.5	149.5
148.3	149.1
148.7	149.9
148.5	149.5
Average 148.5	Average 149.5

The long period of time required for the weak percolate to reach the lower orifice in modified diacolation is again pointed out. In the operation of repercolation some of the experimental details favored a more complete extraction than was previously obtained. The rates of flow of the 500-Gm. and 200-Gm. portions were reduced in proportion to the amounts of drug to conform with the most efficient rate of flow as previously found by Husa and Huyck (93). Because of the slower rates of flow employed, the weak percolate from the 500-Gm. portion could not all be collected at one time. For this reason percolation of the 500-Gm. portion was interrupted for 12 hours between the fourth and fifth 300-cc. portions of weak percolate. The results confirmed the conclusions of the

previous experiment in that the modified repetition diacolation process gave a better extraction of alkaloids of belladonna root than either simple percolation or repercolation.

12. Preparation of Fluidextract of Belladonna Root by Modified Diacolation - In 1934 diacolation was described by H. Breddin (67) (74) and it is taken up under "New Drug Extraction Processes" in the preceding historical review.

In the present investigation a somewhat similar apparatus was devised and assembled. As shown in the photograph (see page 80), a long drug column was obtained by using flanged pipe made of Pyrex glass. The eight sections of glass pipe were joined together by means of U-shaped fittings. Interface joint gaskets made of sulfur-free gum rubber were used at the joints, which were held together by metal joint flanges. The menstruum was forced through the drug by use of compressed air. The dimensions of the tubes were as follows: length 91 cm., thickness of wall 5 mm., internal diameter 2.5 cm., external diameter 3.5 cm. The apparatus differed from the regular diacolation apparatus in the following respects: (a) the menstruum entered the drug at the top of the drug column instead of at the bottom, (b) the drop chamber that enabled adjustment of the rate of flow of menstruum at the beginning of the system was omitted, (c) the tubes were connected to each other by glass U-tubes of the same diameter, which enabled the formation of one long continuous drug column, (d) pressure was supplied from the compressed air line and reduced to the desired pressure by a reducing valve, instead of supplying the pressure by a pump or rubber bulb as directed in regular diacolation.

When powdered belladonna root was moistened in the proportion of 25 cc. per 100 Gm. and when it was packed in sections, the system held 2400 Gm. of moistened drug. As in previous experiments, in order to check on the extraction, the 2400 cc. of fluidextract was collected in two portions of 1200 cc. each and in addition a 1200-cc. portion of weak percolate was collected. The total length of drug column was 936 cm., while the volume of the packed drug was 5120 cc. The time consumed in packing the drug was 6¼ hours. After packing, the system was allowed to stand for 39¾ hours. The menstruum was run in at a pressure of 28 lbs. and after 107 hours or about 4½ days, the menstruum had progressed through six of the eight tubes. The pressure was shut off for a period of 48 hours. After 47 additional hours at 25 lbs. pressure the liquid had progressed through all eight tubes and percolation commenced at the rate of 0.13 cc. a minute for the first 12 hours. By increasing the pressure to 34 lbs. the rate of percolation was increased to 0.30 cc. a minute for the first two portions and up to 1.21 cc. for the weak percolate. The time required to collect the various portions was as follows: first portion of 1200 cc., 74¾ hours; second portion of 1200 cc., 80¾ hours; weak percolate of 1200 cc., 48¾ hours. For the making of 2400 cc. of fluidextract of belladonna root by modified diacolation the total elapsed time was 317¾ hours, while the time consumed by the operator was 10 hours.

As in the previous experiments, in order to check on the extraction, each portion was analyzed for alkaloids and extractive matter. A summary of the average of the analyses of the various portions for alkaloids and

extractive matter is shown in Table XVI.

Table XVI

Preparation of Fluidextract of Belladonna Root by Modified Diacolation.

Assay Results.

Gm. Alkaloids in Various Fractions of Percolate.	
First Reserve-1200 cc.	13.8
Second Reserve-1200 cc.	0.8
Weak Percolate-1200 cc.	0.4
Total 3600 cc.	15.0
Total of First and Second Portions Calculated on 1000 cc.	6.1
Gm. Extractive Matter in Various Fractions of Percolate.	
First Reserve-1200 cc.	168.8
Second Reserve-1200 cc.	167.4
Weak Percolate-1200 cc.	92.6
Total 3600 cc.	428.8
Total of First and Second Portions Calculated on 1000 cc.	140.1

From the results it is concluded that an extremely long drug column and slow percolation under pressure gives a U.S.P. fluidextract of belladonna root (2400 cc. from 2400 Gm. drug). The alkaloidal content of the fluidextract was 6.1 Gm. in 1000 cc. as compared with 5.8 Gm. obtained by simple percolation, 5.7 Gm. obtained by repercolation and 6.3 obtained by modified repetition diacolation using Oldberg percolators.

IV. GENERAL DISCUSSION OF RESULTS.

1. Comparison of the Efficiency of Various Processes of Making Fluidextracts. Although 60 cc. of menstruum per 100 Gm. of drug was used for moistening the drug in simple percolation and U.S.P. XI repercolation as compared with 25 cc. per 100 Gm. drug in the other processes, data on the content of alkaloids and extractive matter in 1000-cc. portions of fluidextract of belladonna root made by the various processes are compared in Table XVII.

Table XVII

Comparison of the Efficiency of the Various Processes as to Extraction of Alkaloids and Extractive Matter in 1000-cc. Portions of Fluidextract of Belladonna Root.

	Gm. Alkaloids in 1000 cc.	Gm. Extractive Matter in 1000 cc.
U.S.P. XI Simple Percolation	5.8	170.4
U.S.P. XI Repercolation	5.7	119.4
Modified Repetition Diacolation Using Oldberg Percolators	6.3	146.4
Modified Diacolation	6.1	140.1

From the table the largest amount of alkaloids was obtained by modified repetition diacolation, while the least was obtained by U.S.P. XI repercolation. As for extractive matter the greatest amount was obtained by simple percolation, while the least was obtained by U.S.P. XI repercolation.

2. Comparison of Time Required in Making Fluidextracts by Various Pro-

cesses. A comparison of the time consumed by the operator and the total elapsed time required in making 1000-cc. portions of fluidextract of belladonna root by the various processes is shown in Table XVIII.

Table XVIII

Comparison of the Time Consumed by Operator and the Total Elapsed Time Required in Making 1000-cc. Portions of Fluidextract of Belladonna Root by Various Processes.

	Time of Operator Required in Hrs.	Elapsed Time Required in Hrs.
U.S.P. XI Simple Percolation	4.2	108.5
U.S.P. XI Repercolation	2.6	176
N.F. II Repercolation	1.8	176
Modified Repetition Diacolation	2.6	176
Modified Diacolation	10*	317.5*

* Time required for making 2400 cc. of fluidextract.

As for time consumed by the operator modified diacolation required the greatest amount of time, while repercolation and modified repetition diacolation required the least. As for time elapsed during the operation, repercolation required the greatest amount of time while simple percolation required the least.

3. The Effect of Method of Packing on the Efficiency of Repercolation. Husa and Huyck (90) found in the case of belladonna root that packing the drug from the top gave more alkaloids in the reserve and less in the weak percolate than when the drug was packed in sections. These results were verified in the case of cinchona by Büchi and Feinstein (41). In reper-

colation, however, the present investigation showed that the method of packing does not influence the efficiency of extraction of the alkaloids or extractive matter of belladonna root.

4. The Effect of Varying the Quantity of Moistening Liquid in Repercolation. Since some of the investigations on repercolation of belladonna root employed 60 cc. of moistening menstruum per 100 Gm. of drug while others employed 25 cc. per 100 Gm. of drug, the results are compared in Table XIX.

Table XIX

Comparison of Different Amounts of Moistening Menstrua on the Repercolation of Belladonna Root.

Gm. Alkaloids in Various Fractions of Percolate.	60 cc. per 100 Gm. Drug.	25 cc. per 100 Gm. Drug.
First Reserve-200 cc.	1.6	2.2
Second Reserve-300 cc.	2.6	1.9
Third Reserve-500 cc.	1.7	1.6
Total 1000 cc.	5.9	5.7
Gm. Extractive Matter in Various Fractions of Percolate.		
First Reserve-200 cc.	19.0	31.9
Second Reserve-300 cc.	37.4	51.7
Third Reserve-500 cc.	60.6	74.2
Total 1000 cc.	117.0	157.8

The results show that the alkaloids of belladonna root were extracted more rapidly when a smaller amount of moistening menstruum was used; also, the smaller amount of moistening menstruum used resulted in a more

efficient extraction of the extractive matter of belladonna root. These results are in general agreement with the results of Husa and Yates (92) concerning the effect of different amounts of moistening menstrua on the percolation of belladonna root.

In the employment of the U.S.P. XI repercolation process for making fluidextract of belladonna root there is always 150 to 250 cc. of weak percolate left over after the collection of the last reserve. In previous work Husa and Huyck (39) recorded 40 to 55 cc. of weak percolate left over in the manufacture of fluidextracts of belladonna root, nux vomica and cinchona by the U.S.P. X repercolation process. In the N.F. II process, however, over 100 cc. of fresh menstruum had to be added in order to collect the last reserve. This irregularity is caused by the different amounts of weak percolate collected from the second portion of the drug which in turn is used to extract the third portion of drug. The amounts of weak percolate collected from the second portion of the drug in the U.S.P. and N.F. repercolation processes of the past and present are: U.S.P. IX, 800 cc.; U.S.P. X, 800 cc.; U.S.P. XI, 1000 cc.; N.F. II, 650 cc.; N.F. III, 650 cc.; N.F. IV, not specified; N.F. V, not specified; N.F. VI, 1000 cc. Although the last 200 cc. of weak percolate contains 0.04 Gm. of alkaloids or 0.7% of the alkaloids present in the finished fluidextract, this portion of percolate does not run through the last portion of drug until after the third reserve has been collected. Consequently, the alkaloids present in the last portion of weak percolate never reach the finished fluidextract. Since this investigation shows the extra 200 cc. of weak percolate is useless, the 800 cc. of weak per-

colate specified by U.S.P. X is the satisfactory amount to be collected.

5. Comparison of the Efficiency of Various Forms of Percolators. In the study of the efficiency of extraction using various forms of percolators, the results with belladonna root were found to be in general agreement with the results of Büchi and Feinstein (41) on cinchona in that the form of percolator did not seem to make any great difference in the extraction of alkaloids or extractive matter. This general conclusion was confirmed in a later experiment, for it was found that the Oldberg percolators were practically as efficient as the tubes in making fluidextract of belladonna root by modified repetition diacolation.

6. Modified Repetition Diacolation. Modified repetition diacolation is a simpler process than the U.S.P. repercolation process in that it divides the drug into three equal portions and collects the same amount of reserve and weak percolate from the first two portions of drug. The factors involved in modified repetition diacolation that appear to be responsible for more efficient extraction than in repercolation are: (a) the proportion of the amount of reserve collected to the quantity of drug used in the first two portions, (b) fresh menstruum is required all through the process after the weak percolates have been used. The idea of dividing the drug into equal portions in repercolation is not new, since it was advocated by Hallberg (14), Cripps (20), Musset (22), Kelly (24), Catford (25) and the British Pharmacopoeia of 1914 (31). Because many investigations, including the recent investigation of Büchi and Feinstein (41), have shown that displacement of weak percolate from the drug by water without diffusion is not successful, this procedure was

not attempted in modified repetition diacolation. However, when water was used by H. Breddin (43) and others for this purpose, some of the menstruum could be left in the drug. One thousand grams of fluidextract was prepared from 1000 Gm. of drug, thus leaving a quantity of menstruum in the drug equal to the weight of total extractive matter in the finished fluidextract.

7. Modified Diacolation. Many features of the diacolation process have been known and used for a great many years. In 1862 Lalieu (3) carried out repercolation using a series of cast iron tubes of about the same dimensions as the tubes used in present day diacolation. Instead of applying pressure as in diacolation, the tubes were arranged on an incline so that the liquid would flow continuously from one to the other. For that reason the apparatus was restricted only to drugs readily extracted by water. Catford (25) in 1898 also used a tube of about the same dimensions as the tubes used in diacolation. The tube was cut in four equal segments capable of being connected end to end, and each segment was designed to hold a definite amount of previously moistened drug. By the proper manipulation of the segments a fluidextract was obtained. Cripps (20) divided the drug into four equal parts and without reserving percolates, carried the menstruum through the whole series of percolators. Pressure in percolation was used in a number of ways more than one hundred years ago. Displacement of alcoholic menstruum by water was used many years ago by Squibb. However, our results indicate that a full strength fluidextract of belladonna root can be made by slow percolation of menstruum under pressure through an extremely long column of drug

without resorting to fractional collection of weak percolate from several portions of drug as in repercolation, and without use of heat to concentrate weak percolate as in U.S.P. simple percolation.

V. SUMMARY

In order to compare the efficiency of various extraction processes, fluidextract of belladonna root was prepared by a number of different methods. The relative efficiency of the different processes was determined by comparing the finished fluidextracts as to content of alkaloids and extractive matter, and by noting the time and labor required in each case. The extraction processes studied were as follows: Simple percolation, repercolation, modified repetition diacolation and modified diacolation.

Concerning the yield of alkaloids and extractive matter the processes are arranged in descending order of efficiency as follows: modified repetition diacolation, modified diacolation, simple percolation, repercolation.

As for the actual time of an operator required, modified diacolation required the greatest amount of time, while repercolation and modified repetition diacolation required the least. As for time elapsed during the operation, repercolation required the greatest amount of time while simple percolation required the least.

An investigation of the effect of method of packing on the efficiency of repercolation showed that the method of packing does not influence the efficiency of extraction of the alkaloids or extractive matter of belladonna root.

A study of the effect of varying the quantities of moistening liquid in repercolation showed that the alkaloids of belladonna root were ex-

tracted more rapidly when a smaller amount of moistening menstruum was used; also, the smaller amount of moistening menstruum used resulted in a more efficient extraction of the extractive matter of belladonna root.

From observations of the U.S.P. XI repercolation process it was found useless to collect any more than 800 cc. of the required 1000 cc. of weak percolate from the second portion of the drug, since the alkaloids contained in the last 200 cc. never reached the fluidextract.

In the study of the efficiency of extraction using various forms of percolators, the results with belladonna root were in general agreement with the recently published results of Büchi and Feinstein (41) on cinchona in that the form of percolator did not seem to make any great difference in the extraction of alkaloids or extractive matter.

Modified repetition diacolation is a simpler process than U.S.P. repercolation, because it divides the drug into three equal portions and collects the same amount of reserve and weak percolate from the first two portions of drug. The factor involved in modified repetition diacolation that appears to be responsible for more efficient extraction than in repercolation is the proportion of the amount of reserve collected to the quantity of drug used in the first two portions.

From a study of modified diacolation the results indicate that a full strength fluidextract of belladonna root can be made by slow percolation of menstruum under pressure through an extremely long column of drug without resorting to fractional collection of weak percolate from several portions of drug as in repercolation, and without use of heat to concentrate weak percolate as in U.S.P. simple percolation.

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This dissertation was prepared under the direction of the Chairman of the Candidate's Supervisory Committee, and has been approved by all members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philocophy.

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